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SEPARATION OF SOME PAIRS OF *CIS-TRANS*-ISOMERIC 4-ALKYLCYCLOHEXANE-1-CARBOXYLIC ACIDS BY MEANS OF INCLUSION COMPOUNDS WITH THIOUREA

(Preliminary Communication)

BY

H. VAN BEKKUM, P. E. VERKADE, AND B. M. WEPSTER

(Communicated at the meeting of April 25, 1959)

In connection with synthetic work on some groups of cyclohexane derivatives [1]), performed with the object of ascertaining the constitution —*cis*- or *trans*-isomer— of the compounds in question, for some pairs of *cis-trans*-isomeric 4-alkylcyclohexane-1-carboxylic acids the striking discovery was made that, at least under the experimental conditions employed by us, the *trans*-isomer can and the *cis*-isomer cannot be included in thiourea [2]).

This fact could be successfully turned to account for the separation of the pairs of isomers concerned. Both when starting from mixtures consisting predominantly of *trans*-isomer (obtained by reduction of the corresponding 4-alkylbenzoic acids in an aqueous alkaline medium at elevated temperature with hydrogen under high pressure in the presence of Raney nickel) and when starting from mixtures consisting predominantly of *cis*-isomer (obtained by reduction of the corresponding 4-alkylbenzoic acids in glacial acetic acid with hydrogen under slightly more than atmospheric pressure, using platinum dioxide as the catalyst), the two isomers could be very easily obtained in pure condition and in a good total yield. This new procedure is valuable from the preparative point of view. As a matter of fact, in the present and similar cases application of the classical separation procedures (such as fractional crystallization of the mixture of acids) generally does not produce a satisfactory result in a simple way.

The separation in question can obviously be performed in different ways. We think we may here confine ourselves to a concise and general description of one technique which was highly satisfactory to us.

The mixture of *cis-trans*-isomeric 4-alkylcyclohexane-1-carboxylic acids to be separated is dissolved in a suitable quantity of a hot saturated solution of thiourea in absolute methanol. Upon being cooled to about 0°,

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[1]) See the thesis of H. VAN BEKKUM, which is to appear shortly.

[2]) W. SCHLENK Jr., Lieb. Ann. **573**, 142 (1951).

the inclusion compound crystallizes in the form of beautiful needles, possibly along with thiourea; the crystal form of the latter substance is quite different and its presence can thus be readily recognized. The crystalline material is subsequently collected by filtration. If no clearly perceptible crystallization of thiourea has taken place, the filtrate, after having been heated approximately to boiling, is saturated with thiourea, after which the solution is treated again in the way described above.

The crude inclusion compound thus obtained is dissolved in hot dilute potassium hydroxide solution. Addition of mineral acid to the cooled solution produces the crude *trans*-isomer, which is filtered off and purified by crystallization from a suitable solvent.

The methanolic filtrate is diluted with water. After partial evaporation with a view to the removal of the alcohol, the remaining solution is cooled and mineral acid is added, upon which the crude *cis*-acid separated is obtained by filtration. The acid is finally purified by crystallization from a suitable solvent.

If desired, the whole of the procedure described above may of course be repeated with the combined acid fractions isolated from the different mother liquors. The separation of the *cis-trans*-isomers thus becomes practically quantitative.

In the table below we list the melting points of four pairs of *cis-trans*-isomeric 4-alkylcyclohexane-1-carboxylic acids obtained in the above way, as well as the melting points of the corresponding *p*-bromophenacyl esters.

4-R-cyclohexane-1-carboxylic acid	acid		*p*-bromophenacyl ester	
	cis	*trans*	*cis*	*trans*
	m.p.	m.p.	m.p.	m.p.
R = ethyl	22.5–23.5°	49.5–50°	84–84.5°	112.5–113°
isopropyl	40–41°	94–94.5°	85–85.5°	108–108.5°
t-butyl	118–118.5°	175–176°	102–102.5°	115–115.5°
neopentyl	64–64.5°	120.5–121°	127–127.5°	111.5–112°

With regard to the formation of the inclusion compounds in question and the contrast in the behaviour of the *cis*- and the *trans*-isomers the following remarks may be made:

1. According as the size of the alkyl group increases, the stability of the inclusion compound of the *trans*-isomer was found to increase. In fact, the solubilities of *trans*-4-t-butyl-, *trans*-4-isopropyl-, and *trans*-4-ethylcyclohexane-1-carboxylic acids in a saturated solution of thiourea in methanol at 20° proved to be approximately as 1:3:18; that of *trans*-4-ethylcyclohexane-1-carboxylic acid is about 5.0 g/100 ml.

2. The molecular ratio of thiourea and *trans*-acid in the inclusion compounds is of course easily determined by titration of the acid and,

as is readily understood, appeared to differ little in the three cases just-mentioned: in fact, the ratio found was 5–5.5:1.

3. The above-mentioned difference in behaviour between the *cis*- and the *trans*-acids in question is stressed by the fact that the methyl esters of *cis*- and *trans*-4-t-butylcyclohexane-1-carboxylic acid and also *cis*- and *trans*-1,4-di-t-butylcyclohexane – we mention only a few examples – both form an inclusion compound with thiourea. It is thus natural to seek the cause of the different behaviour of the acids in the presence of a carboxyl group. In this connection an observation of NICOLAIDES and LAVES [1]) may be of interest. In fact, these investigators found that fatty acids are included in urea in the form of dimers, *i.e.* with association *via* the carboxyl group. In the *trans*-4-alkylcyclohexane-1-carboxylic acids the carboxyl group and the alkyl group both have an equatorial position; the molecules of these compounds accordingly have a „linear" structure and will undoubtedly also as dimers fit in the thiourea channel. In the case of the *cis*-4-alkylcyclohexane-1-carboxylic acids, on the other hand, axial position of the carboxyl group, next to equatorial position of the alkyl group, plays a part which depends on the character of the alkyl group, but which is no doubt always important; especially in the case of the *cis*-4-t-butylcyclohexane-1-carboxylic acid involved in the present work the said position of the carboxyl group is highly predominant. It would seem quite plausible to us that with such a position of the carboxyl group there will be no room for inclusion of the acids in question as dimers in the thiourea channel. We are, however, quite prepared to admit the speculative character of this attempt at explanation of the difference in behaviour between the isomers in question.

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[1]) N. NICOLAIDES and F. LAVES, J. Am. Chem. Soc. **80**, 5752 (1958).

ACID FUCHSIN AND URANYL NITRATE IN STAINING CHROMATOGRAMS OF PHOSPHATIDES

BY

H. G. BUNGENBERG DE JONG AND G. R. VAN SOMEREN

(Communicated at the meeting of April 25, 1959)

1. *Principle of the staining reaction with acid fuchsin and uranylnitrate*

For several years (1952) a test for spots of total egg-phosphatides on chromatographic paper (WHATMAN no. 1, or SCHLEICHER and SCHÜLL no. 2043 B) has been realised. The paper is immersed in an acid solution of acid fuchsin and uranyl nitrate and after some time the spots stain red, whereas the paper itself hardly takes a colour.

We were led to this staining mixture (and analogous with other acidic dyes) by considering that the individual phosphatides in total egg-phosphatides (lecithin, kephalin, their eventual lyso-products and sphingomyelin) all belong to phosphatides which have in their molecule one positively and one negatively charged group.

From former investigations on "Tricomplex-Systems", [1]) it seemed possible that spots of the said phosphatides on paper will stain with appropriate dyes (cationic or anionic) in the presence of a well chosen not (or weakly) coloured ion (anion or cation), when the latter has a sufficiently strong affinity to the phosphatide and on the other hand has only a weak affinity to the dye.

Of the two possibilities, the use of a cationic dye plus appropriate anion seems not very promising. First, because basic dyes in general stain the paper itself more or less strongly and thus give an undesirable background colouration. In the second place, it is difficult to find an anion with a large affinity for the positive group of the phosphatide and at the same time with a small affinity for the dye cation.

Thus our choice fell on acid dyes and an appropriate cation. Former investigations on "Reversal of charge spectra" [2]) had revealed that among inorganic cations the uranyl-ion has by far the greatest affinity for the phosphate group, whereas this cation has not at all a very prominent affinity for carboxyl- or sulphate-groups. For realising staining of spots of total egg-phosphatides one should therefore try a mixture of an acid dye and an uranyl-salt.

[1]) For a description of this kind of colloid systems see H. R. KRUYT, Colloid-Science II, Elsevier Publishing Company, inc. 1949, Chapter X, section 6, by H. G. BUNGENBERG DE JONG.

[2]) For a survey of the result, see in A. R. KRUYT, Colloid Science II, loc. cit., Chapter IX, section 2, by H. G. BUNGENBERG DE JONG.

The first combination investigated, viz. Indigocarmine + uranyl nitrate had a complete success. Without $UO_2(NO_3)_2$, Indigocarmine did practically not stain the spots (very concentrated spots only weakly), whereas in the presence of $UO_2(NO_3)_2$ a strong colouration was obtained.

Also other acidic dyes give in the presence of $UO_2(NO_3)_2$ the expected "tricomplex staining" (e.g. Red Ponceau and Metanil Yellow), but the most satisfactory combination hithertoo obtained is that with acid fuchsin + $UO_2(NO_3)_2$.

So far, our expectation of the possibility of tricomplex staining of total egg-phosphatides came true in 1952.

Later we succeeded in obtaining, with $Be(OH)_2$ impregnated paper and 95 % ethanol as mobile phase, a crude separation of total egg-phosphatides into elongated spots, both giving the reaction for phosphate groups. The upper showed the reaction for choline, the lower gave the ninhydrin reaction. Though this chromatographic separation was far from ideal (tendency to tail formation) it was important that both spots were stained red with Acid Fuchsin + $UO_2(NO_3)_2$. We had not expected otherwise, for the tricomplex staining should not be specific for one component of total egg-phosphatides, but for all (see above).

In the past year, chromatography of phosphatides has been taken up anew, in this laboratory, with the collaboration of G. J. M. Hooghwinkel and J. Th. Hoogeveen.

Modern methods allowing the separation of lecithin, lysolecithin, kephalin, lysokephalin and sphingomyelin (silica impregnated paper; di-isobutylketon – acetic acid – H_2O mixtures as mobile phase) have been applied and it appeared that spots of the above-mentioned five components all are stained with the Acid Fuchsin – $UO_2(NO_3)_2$ staining solution.

2. *Particulars of the staining method with acid fuchsin + uranylnitrate*

We first give the composition of the staining solution and washing liquid used in this laboratory, and subsequently the reason for this choice.

The staining solution is made as follows:

100 ml 0,1 N HCl
100 ml 2 % uranyl nitrate 6 aq. in water
10 ml 0,2 % Acid Fuchsin in water
790 ml distilled water.

Chromatograms need not be stained longer than $\frac{3}{4}$ hour, to make spots of low concentration visible.

The chromatogram is blotted with filterpaper and is washed twice (5–10 min) with the following liquid

100 ml 0,1 N HCl
100 ml 2 % uranyl nitrate 6 aq.
800 ml H_2O.

After one more blotting, the strips are dried. We prefer rapid drying with a "Föhn" (electric hairdryer), because intensely coloured spots, when present, tend to bleed when drying at room temperature. This bleed, forming artefacts on the paper surrounding the spot, is prevented by rapid drying. The dried chromatograms may be kept unchanged (when not constantly exposed to light).

It is not advisable to prepare large quantities of the staining solution, for, despite the low pH and the presence of uranyl nitrate, moulds tend to develop in it after standing a longer time at room temperature. Mould growth in the 0,2 % acid fuchsin stock solution is prevented by keeping it in the refrigerator ($\sim 5°$ C).

As to the acidity of the staining solution the following may be remarked. A slight acidity must in any case be present to prevent hydrolysis of uranyl nitrate. In slightly alkaline medium the uranyl-ion ceases to exist (formation of uranate) and thus the staining according to the "tri-complex mechanism" is no longer possible [1]).

We prefer, however, a lower pH of the staining solution than necessary to prevent the above mentioned hydrolysis. It has been found that this lessens the slight background coloration of the paper, and thus enhances the contrast between weakly coloured spots and surrounding paper. One may for instance use 0,1N – 1N acetic acid, but the contrast on silica impregnated paper is still better with 0,01N HCl.

The intensity of staining is but small at very low concentrations of uranyl nitrate, it increases with increase of the concentration of $UO_2(NO_3)_2$, but it is not useful to use a higher concentration than about 0,2 % (the concentration in the above formula for the staining solution).

It will be clear that the washing liquid should have the same HCl and $UO_2(NO_3)_2$ concentration as the staining solution.

The concentration of acid fuchsin may be varied between wide limits. With higher concentration, for instance 0,02 %, staining is very rapid, but as the paper is now soaked by the red solution, it must be washed briefly with the washing liquid to make spots of low colour intensity visible (corresponding to a low surface concentration of the phosphatide in the spot). Such a 0,02 % staining solution may be used with profit as an aid in column fractionation. A falling drop is collected on a piece of unprepared chromatographic paper and after rapid drying with a Föhn, the paper is put in the staining solution. We can thus rapidly control

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[1]) This explains the fact that a chromatogram which has been stained in acid medium looses gradually all colour in running tapwater (slightly alkaline). The now uncoloured strip can be stained anew in the acid staining solution. The maximum colour to be reached is now as a rule lower than originally, because of a certain loss of phosphatide. Such losses are far greater when rocking a chromatogram before staining some time with destilled water (now neither protecting $UO_2^{\cdot\cdot}$-ions, nor weakly protecting Ca-ions being present to prevent considerably swelling of the phosphatide, Compare section 3).

whether the liquid still contains phosphatide or practically no longer does so.

For staining chromatograms however, we prefer the ten times lower concentration (0,002 %) as in the above formula for the staining liquid. It is true, the rate of staining is now much smaller, but on the other hand two advantages are booked. First the red colour of the staining fluid soaking the paper is now sufficiently small that weakly coloured stains can be discerned by taking the chromatogram out of the solution. The course of the staining can thus be followed without the need of washing out. In the second place it has appeared that with silica impregnated paper the slight background colloration (after washing out) is less than after staining with the more concentrated acid fuchsin solution.

It is even not strictly necessary to wash out the chromatogram stained with the 0,002 % acid fuchsin solution. When blotted on filter paper and dried with a "Föhn", weakly stained spots are still visible, though the background is now much stronger coloured.

As to the sensitivity of the staining method investigations have been performed in this laboratory by G. J. M. Hooghwinkel. Spots were brought on chromatographic paper with a micropipet of solutions (chloroform + methanol = 4 : 1) of lecithin or kephalin (column fractions) of decreasing concentration. After drying they were stained with the 0,002 % acid fuchsin containing solution. It has been found that after drying, both with lecithin and kephalin, the spots from 0,03 % solution are visibly coloured, the spots from 0,01 % were sometimes visible, sometimes not. The limit thus lies at about 0,02 %. This limit applies for both papers investigated (Whatmann no. 1 and Schleicher and Schüll no. 2043 B), and is not altered when these papers have been impregnated beforehand with silica. Remarkably enough the limit remained about the same when the stained strips were not washed out and thus a distinctly red background remained.

3. *Some observations concerning the mechanism of the staining with Acid Fuchsin + uranyl nitrate*

When a streak of total egg-phosphatides on a slide, in contact with a drop of distilled water, is observed under the microscope, one perceives the temporary formation of "myelin tubes". These are an outgrowth of the smectic phase, but in distilled water they soon lose their distinct double refraction between crossed nichols because of swelling. After some time the peripheral myelin tubes tend to lose their characteristic shape and to change into drops. When the streak of phosphatide is brought in contact with a salt solution (e.g. $CaCl_2$) the myelin tubes are much more double refracting and have not such a great tendency to swell up to drops. This change in character is most prominent with urany lnitrate. Already at low concentration the negative surplus charge of the phosphatide is taken away and this lowers swelling. At the reversal of charge

concentration, the smectic phase is most condensed and growth of myelin tubes is practically suppressed. At higher concentrations the charge is reversed to the positive sign and myelin tubes are formed anew. They, however, have no tendency to swell to drops.[1])

The uranyl nitrate concentration used in our staining formula is high enough to form such dense myelin tubes.

When a streak of egg-phosphatides is brought in contact with the acid fuchsin staining solution it is observed that once more this dense type of myelin tubes is formed, and that gradually they become very deeply coloured. We are thus informed, that the staining is not an adsorption of the dye at the surface of a phase (smectic phase), but that the dye is taken up into the interior of this phase.

When the above experiments are repeated with slides covered with a thin layer of phosphatide (slides are dipped into a 3% solution of phosphatides in ether, the thin layer being formed after evaporation of the solvent), no abundant myelin growth is observed in contact with water, salt solutions or staining solution.[1])

There is nevertheless a distinct difference in behaviour of the layer in contact with water and with the other solutions. With water the layer takes on a sirupy consistency. When a needlepoint is moved over the slide no incision becomes visible. In contact with the other liquids a more or less distinct incision is formed and the two margins may recede from one another, making the cleft wider. At the margins here and there small dense myelin tubes may arise. We see here that a thin layer does not swell considerably in contact with appropriate salt solutions, on the contrary it may show a tendency to contract.

This is thus a parallel to the behaviour of myelin tubes growing from a streak of phosphatide, discussed above. Indeed one must assume that also thin layers in contact with water consist of the smectic phase, the latter swelling up in contact with water to syrupy consistency, but assuming a dense character in contact with the salt or staining solutions.

The above will serve to explain the following facts. When spots of different phosphatide concentrations are brought on paper and after drying are stained, spots corresponding to low concentrations near the limit of visibility (0,1 %; 0,03 %) are stained to their maximum intensity in $\frac{3}{4}$ hours, spots corresponding to large concentrations (3 %; 1 %), though already intensely coloured in $\frac{3}{4}$ hours, continue to deepen in intensity of staining overnight. This difference is readily explained by considering that in 3 % and 1 % spots the phosphatide is present in a relatively thick layer on the paper fibers – thus comparable to streaks on the slide – and it will take a much longer time before the interior of the smectic phase is stained. The phosphatide in the low concentrated spots

[1]) H. G. Bungenberg de Jong and J. L. L. F. Hartkamp, Protoplasma 31, 550 (1939).

(0,1 % and 0,03 %) is present as a much thinner layer — comparable to a thin layer on the slide — and thus the spots are stained through in a much shorter time.

A further difference in behaviour of phosphatide spots of high and low concentration is as follows: When a paper strip with spots corresponding to different phosphatide concentrations is dried and stained overnight, and the wet strip is then blotted between filterpaper applying some pressure, it appears that the high concentration spots may give of red specks on the filter paper, whereas this does not occur with the spots corresponding to low concentrations. This difference is once more explained by considering that at 3 % and 1 % the phosphatide is present as a thick layer and thus a considerable myelin tube outgrowth will arise, a number of tubes emerging above the surface of the paper. By blotting with filter paper applying some pressure these stained myelin tubes break of and adhere to the filterpaper. With low concentrations — the phosphatide layer now being thin — no abundant myelin growth occurs at staining and thus no red specks appear on the filter paper with which is blotted.

4. *Staining solution containing Acid Fuchsin, uranylnitrate and Brilliant Green*

It is known that in total Soybean phosphatides are present lecithin, kephalin and inositolphosphatides. As the first two have one positive and one negative charge, their corresponding spots on a chromatogram should stain with acid fuchsin + $UO_2(NO_3)_2$ at pH2. The inositolphosphatides, which are reported to contain uncompensated phosphate groups should not stain with acid fuchsin + $UO_2(NO_3)_2$ at pH2, but with a suitable basic dye.

At present chromatography of Soybean phosphatides is studied in this laboratory in collaboration with G. J. M. Hooghwinkel and J. Th. Hoogeveen. Among other detection methods the chromatograms are also stained with a mixed solution of Acid Fuchsin and Brilliant Green, containing Acid Fuchsin (0,006 %), Brilliant Green (0,01 %), $UO_2(NO_3)_2$ (0,2 %) and HCl 0.01N. [1]) Preliminary results confirm our expectation. With a crude Soybean phosphatide (commercial sample) two distinct red spots and a third small red spot were obtained with same Rf values as egg-lecithin, egg-kephalin and egg-lysolecithin respectively, further green spots, some of which showed a phosphate reaction. Other green spots, however, did not react with the phosphate reagent. As to the latter fact one must bear in mind that substances with an acidic group other than

[1]) The freshly prepared mixed staining solution must stand for several hours—preferably overnight—before it is used for staining chromatograms. During this time the original very dark solution brightens up slowly to a red colour not very different from the Acid Fuchsin solution of the same strength in the same medium (uranyl-nitrate; 0,01 N HCl) without brilliant green. Reason for this brightening up is the slow transition at pH2 of the deeply coloured Brilliant Green into a slightly yellowish-green solution. This transition is reversible at increase of the pH.

phosphate should also stain green, provided the acidic group is still sufficiently ionised at pH2. Further details concerning the chromatograms must be postponed to a later communication.

In the following we will confine ourselves to the method itself.

It must first be remarked that possibly Brilliant Green is not yet the best choice of a basic dye in our mixed staining solution, though it gives a good contrast staining.

The ideal basic dye would be one, which when present alone at pH2 and in the presence of $UO_2(NO_3)_2$, does not stain the lecithin and kephalin spots at all. With Brilliant Green this ideal situation is not reached, these spots taking a not very intense green colour. Possibly this is due to a weak tricomplex staining of the type basic dye + anion, the staining solution containing Cl^- and NO_3^- and perhaps in small concentration uranyl containing complex anions. For this reason the Acid Fuchsin concentration in the above mixed staining solution is thrice as strong as in that used in section 2 to force in any case tricomplex-staining of the type phosphatide + acid dye + UO_2-ion.

The choice of a low pH is necessary with the available chromatographic papers (WHATMANN no. 1 and SCHLEICHER and SCHULL no. 2043 B) to prevent strong background coloration of the paper. Obviously these papers contain substances with carboxyl-groups, which at pH2 are practically no longer ionised.

The complete staining of the chromatogram with the mixed staining solution takes a longer time than with the staining solution containing only acid fuchsin. The reason is that the green spots develop slowly. After $\frac{3}{4}$ hour they just become visible and only after one night they have reached their maximum intensity. The strips must be washed thoroughly with 0.01N HCl containing 0.2 % $UO_2(NO_3)_2$. This washing (twice during 10 minutes) is necessary to remove as much as possible of the brilliant green soaking the paper. When not washed out, the paper background will turn green at drying.

It is further advisable to finish the washing with 1N acetic acid during a few minutes. This removes most of the HCl. When this washing is omitted and the chromatogram is dried with a Föhn, the green spots may turn yellow and then become difficultly visible (increase in concentration of HCl in the paper). Though by exposure to NH_3 vapour the yellow spots are turned green anew, it is simpler to avoid the complication by the above mentioned final washing.

The dried chromatogram now shows red and green spots on a still light green paper background.

5. *Background coloration and diverse classes of contaminating substances in chromatographic paper*

In the preceding section we mentioned already that brilliant green gives a marked background coloration to the paper, though it can be kept

low by the measures taken (working at pH2 and thoroughly washing at pH2). We were struck by the fact that when using our combined staining solution a zone directly adjacent to the frontline is always coloured more or less blue-green. The same blue staining at the front is obtained when we use a staining solution containing Brilliant Green and no Acid Fuchsin. Obviously one or more contaminating substances present in the paper, which can be stained at pH = 2 with Brilliant Green, move upward with the front. We will in the following design this class of substances with "brilliant green substances".

Experiments have been made with much simpler liquids as used in chromatography of phosphatides with strips of chromatographic papers in the absence of phosphatides on the paper. It has been found that many, but not all liquids, transport "Brilliant Green substances" upward with the moving front. As an example we quote here the results obtained with *n*-propanol water mixtures. When we take water, 10 vol % or 20 vol % propanol as mobile phase, no staining is obtained with Brilliant Green directly under the frontline. With 30, 40, 50, 60, 70, 80, 90 and 100 % vol %, this zone was stained green, the maximum intensity lying at about 40–50 vol % propanol.

An interesting fact was observed with liquids which transport Brilliant Green substances with the front. First we let the liquid ascend to the height of a cm, then dry the strip and let the same liquid ascend for a second time but now to a lesser height, for instance to a -1 cm and go on with this procedure several times. Finally the dried strip is stained. We then obtain in general as much green bands as fronts have been present in the paper. As a rule the first band has the greatest colour intensity, the following bands decrease in intensity. It follows that not all Brilliant Green substances are transported with the front, but that a considerable fraction remains in the paper, part of which is transported with the second, the third and so on ascension of the mobile phase in the paper.

"Brilliant Green substances" are not the only class of substances which contaminate the paper and which are transported upward with suitable liquids with the front. When we let various water-*n*. propanol mixtures ascend in strips and apply after drying the Schiff reaction, it is found that no reaction is obtained when we had used H_2O, 10 or 20 or 30 vol % propanol, but that a distinct positive reaction is obtained just below the frontline after ascension with 40, 50, 60, 70, 80, 90 and 100 % propanol. Thus aldehydic substances are present in the paper and they are transported with the front by much the same propanol-water mixtures as the above-mentioned Brilliant Green substances.

We may of course ask if Brilliant Green substances and aldehydic substances are really two distinct classes or if only one class is present, possessing ionized acid groupings and aldehydic groupings at the same time. To answer this question a long dry strip was brought in a paper

electrophoresis apparatus, both ends of the strip dipping in 67 vol % propanol containing a borate buffer (100 ml borate buffer of pH. 9, 100 ml H_2O and 400 ml *n*-propanol). Some time after the two moving fronts had met near the middle of the paperstrip, the electric field was applied during a sufficient time. After drying the strip was cut lengthwise in two halves. With one half the Schiff reaction was performed. A violet band appeared at or very near the original meeting line of two fronts. The other half of the strip was stained with Brillant Green at pH2. A green band appeared lying markedly at the anode side of the original meeting line of the two fronts. We must thus conclude that Brilliant Green substances and aldehydic substances in the paper are really two different classes, which can be separated by electrophoresis.

It is not quite certain but it seems probable that aldehydic substances can be transformed into Brilliant Green substances by oxydation. This might explain the following observation. When a liquid has ascended in the paperstrip, which moves both aldehydic and Brilliant Green substances upward with the front and after drying at room temperature the strip is cut lengthwise in two halves, while one of the halves is heated (for instance 1 hour at 100° C), the two halves show a distinct difference in intensity of staining with Brilliant Green at the front. With the heated strip the intensity of the green band is much stronger. But also the slight green coloration of the whole strip has increased by the previous haeting. This need not be in contradiction with the supposed formation of Brilliant Green substances at the cost of aldehydic substances. The latter class of substances shows namely the same phenomenon at repeated ascent of the mobile phase to every time lower heights, as described above for the Brilliant Green substances. At each front which was originally present in the paper we find a very distinct Schiff reaction. Thus only part of the aldehydic substances is moved upward with the front at the first ascension, the rest remaining in the paper. Part of this rest is moved upward at the second ascension and so on. Thus in the heated half of the strip the increased staining of the paper itself may be explained by oxydation of aldehydic substances to Brilliant Green substances.

Apart from Brilliant Green substances and aldehydic substances, we found that a third class, namely of ninhydrin positive substances is present in chromatographic paper. Here we have the same general properties as described above for the two other classes.

With a suitable liquid, we find a positive ninhydrin reaction at the front. Here too part of the ninhydrin positive substances is moved upward with the front, part remains in the paper. At a second ascension part of the rest is moved with the front and so on. That these ninhydrine positive substances form an apart class distinct from Brilliant Green substances and aldehydic substances, appeared from the fact that with certain liquids the first are moved, the two other practically not and vice versa. We have for instance compared water 90 % methanol 90 % ethanol,

90 % *n.* propanol and 90 % n-butanol. The most intense ninhydrin reaction was obtained at the front with water, it was less intensive with 90 % methanol, still weaker with 90 % ethanol; hardly present with 90 % *n.* propanol and not visible with 90 % *n.* butanol. The sequence of the intensity of the Brilliant Green staining at the front was, however, just the reverse. The intensity with water was practically nil and it increased in the order 90 % methanol – 90 % ethanol – 90 % propanol and was very intense with 90 %-butanol.

For the purpose of using basic dyes in staining solutions (as in section 4) the presence of the so-called Brilliant Green substances, that is of acidic substances, is a nuissance and we have tried to free the paper from these. Various treatments have been tried, in many cases the movable Brilliant Green substance was increased by the treatment, in many other cases no noticeable effect was seen. A much lower intensity of staining at the front and a practically absent background staining of the paper itself was obtained by soaking the paper during a couple of days in 85 or 95 % ethanol.

Summary

1. A method has been worked out to stain on chromatograms of phosphatides-mixtures only those spots which correspond to phosphatides carrying one negative charge (fosfate group) and one positive charge (choline or ethanolamine-group) in the molecule.

2. Guided by results of former investigations on "Tricomplex Systems", it seemed possible, that phosphatides belonging to the above class, will become stained in watery medium, when an appropriate dye and an appropriate inorganic ion of opposite sign is present. Reasons have been given why the combination acid dye + appropriate cation opens much wider perspectives then the combination basic dye - appropriate anion (background colouration of the paper).

3. It has been found that the expected staining indeed occurs with. dilute solutions of a number of acid dyes in the presence of uranyl ions

4. The most satisfactory staining solution hithertoo worked out contains Acid Fuchsin (0,002 %), uranyl nitrate (0,2 %) and HCl 0,01N., the reasons for this choice having been given in detail. A satisfactory staining is obtained in $\frac{3}{4}$ hours. The chromatograms are then washed with 0,01 N HCl containing 0,2 % uranyl nitrate and dried. The dried chromatograms may be kept unchanged (when not exposed to light). The red spots stand out on the almost uncoloured surrounding paper. In chromatograms of total egg phosphatides we could thus stain red lecithine, kephaline, sphingomyeline and eventually present lysolecithin and lysokephalin.

5. The mechanism of the staining reaction has been discussed, it leading to the result that the said phosphatides occur as a smectic phase in the spots and that the Acid Fuchsin is taken into the interior of this smectic phase.

6. In the case of phosphatide mixtures containing next to phosphatides of the sub 1. mentioned class, other phosphatides with only acid character, it seems possible to stain the corresponding spots in two different colours, using a mixed staining bath which besides Acid Fuchsin and uranyl nitrate contains a suitable basic dye.

7. Preliminary experiments with total Soybean phosphatides show that this can be realised in principle by staining the chromatograms with a dye solution containing 0,006 % Acid Fuchsin, 0,01 % Brilliant Green, 0,2 % uranyl nitrate and 0,01N HCl. After staining, red and green spots are present. For particulars the text must be consulted.

8. Chromatographic paper contains at least three different classes of contaminating substances which may be partly transported upward with the moving front, partly remain in this paper, and give rise to background reactions.

We may discern substances which stain with brilliant green at pH2, a second class which shows the Schiff reaction and a third class which shows the ninhydrin reaction.

9. It has been shown that the first named class of substances has acidic nature (moves in the electric field to the anode). Its presence in chromatographic papers is not desired, as they give rise to more or less strong general background colouration of the paper with basic dyes. It is difficult to free the paper wholly of this class of substances. To diminish as much as possible this background colouration the mixed staining solution contains 0,01 N HCl.

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ON HELICITIC STRUCTURES AND THE OCCURRENCE OF ELONGATE CRYSTALS IN THE DIRECTION OF THE AXIS OF A FOLD

BY

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(Communicated by Prof. F. A. VENING MEINESZ at the meeting of May 30, 1959)

Introduction

In a recent paper the author (1958) developed a theory of schistosity based on the elastic anisotropy of the implied minerals. The opinion was expressed that the same mechanical principles could be of value for the explanation of other phenomena encountered in geology. One of these was the occurrence of so-called helicitic structures, of which TURNER and VERHOOGEN (1951, p. 504) gave the following definition [1]: "The term helicitic structures is applied to curved or contorted lines of inclusions (graphite, ironore, mica, and so on) preserved within coarse crystals (phenocrystals [2])) of minerals such as albite, biotite, staurolite, and chlorotoid".

From the present discussion we will preclude those helicitic structures in which the deformation was precrystalline. But besides structures in which the deformation was paracrystalline we will also discuss analogous structures in which postcrystalline deformation occurred. As, in our opinion, this type of structures is genetically closely related to the paracrystalline deformation type, and generally is also discussed together with the pre- and paracrystalline deformation structures, the following extension of the mentioned definition is proposed: ". . . and to structures in which a crystal has been rotated with regard to its surroundings (but otherwise need not have been deformed), the rotation becoming clear from curved or contorted lines preserved in the enclosing material". The form of the rotated crystal is not defined; it may range from a cube to geometrically irregular forms. A beautiful example of such a structure, in which the rotated crystal has the form of a parallelepiped, is given by SCHUILING [3]) (1957, fig. 3). Illustrations of the paracrystalline deformation type are given e.g. by BAILEY (1923) and READ (1956).

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1) This definition goes back to Weinschenk.

2) TURNER and VERHOOGEN here used the term porphyroblasts.

3) We feel greatly indebted to Mr SCHUILING, who drew our attention to these remarkable structures, showing us his fine thin slides.

Current opinion on the origin of helicitic structures of the para- and posterystalline deformation type assumes that a shearing movement occurred parallel to the schistosity plane, so that the phenocrystal "was being rolled along, and was growing larger, like a snowball, during the process" (BAILEY quoting FLETT). This mechanism, however, cannot be made compatible with the theory of schistosity, developed by the author, which formulates that the schistosity planes are at right angles to the direction of maximum strain, which again implies that no shearing along the schistosity plane can occur.

In the following pages it is argued that helicitic structures of the para- and posterystalline deformation types can be explained simply by a compression at right angles to the schistosity plane. For this explanation again use is made of the theory of elastic anisotropy, this time considering not only the inherent elastic anisotropy but also the influence of the shape of the involved crystals.

Again an analogy will appear to exist between mechanical orientation and orientated crystal growth. This offers the explanation for another feature frequently encountered in geological structures, viz. the occurrence of elongate crystals directed with their longer axes in the tectonical *b*-direction (the folding axis). Dr. H. J. ZWART from the Geological Institute at Leiden was kind enough to draw our attention to the circumstance that even mica may form such crystals. An example of this is given in fig. 1, which shows a gneiss taken at right angles to the tectonic *b*-axis, and to the *a*-axis (i.e. the axis in the *s*-plane at right angles to the *b*-axis).

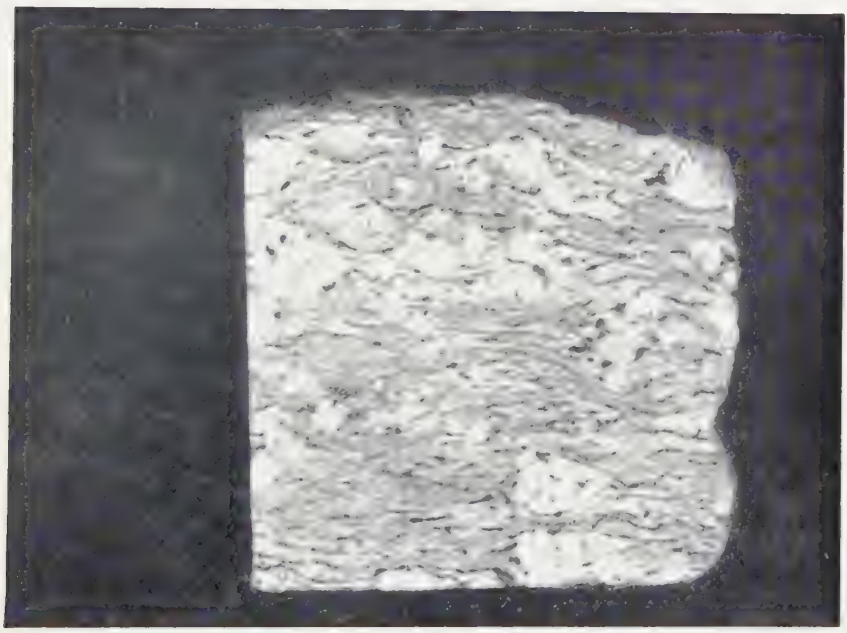
Some experiments are described which illustrate the origin of a helicitic structure and in general confirm the validity of the involved principles.

Elastic anisotropy due to form: mechanical effects

The mentioned theory of schistosity related the origin of this phenomenon to mechanical rotation and orientated (re)crystallization of the implied minerals, due to the anisotropic character of these minerals.

Let us first review the mechanical principles involved. By imagining a spherical, elastic anisotropic inclusion in a homogeneous material and examining the deformation the inclusion undergoes when this material is stressed in one direction, it was concluded that a rotation of the inclusion occurs, to begin with infinitesimally, but which by plastic deformation obtains finite proportions. The explanation for this behaviour is that for a crystal, loaded in a direction which is not parallel to one of the crystallographic axes, we have to consider the elasticity moduli of the type s_{hj} ($h = 1, 2, 3$; $j = 4, 5, 6$), which moduli represent a relation between the principal stresses σ_1, σ_2, σ_3 and the angular deformations ε_4, ε_5, ε_6. Obviously these strains cannot develop without giving rise to asymmetric shearing stresses in the surrounding material and in the inclusion. This causes the rotation. It further could be shown that this rotation was such that the

(a)



(b)

Fig. 1. Gneiss from the Aar Massif.
a. At right angles to the *b*-axis; the mica appears as dots.
b. At right angles to the *a*-axis; the mica shows a layered aspect. Also the felspars are elongate into the *b*-direction.
(*Collection Mineralogical-Geological Institute, Utrecht*).

crystallographic direction, to which the greater of the moduli s_{11}, s_{22}, s_{33} belongs, is turned towards the direction of maximum stress, and in case of plastic flow, turned into this direction. With this orientation the mixed moduli vanish, that is to say, with crystals of the higher symmetry classes.

No serious attention was paid, however, to elastic anisotropy due to an anisotropy of form of an inclusion [1]). Actually it was Mr. G. W. VELTKAMP who mentioned this side of the problem to us. We will try to evaluate the influence of the form as follows.

Suppose a cubic, isotropic particle is surrounded by a material of lower elasticity constants and imagine the particle to be enveloped by a sphere whose center coincides with the center of gravity of the cube (fig. 2).

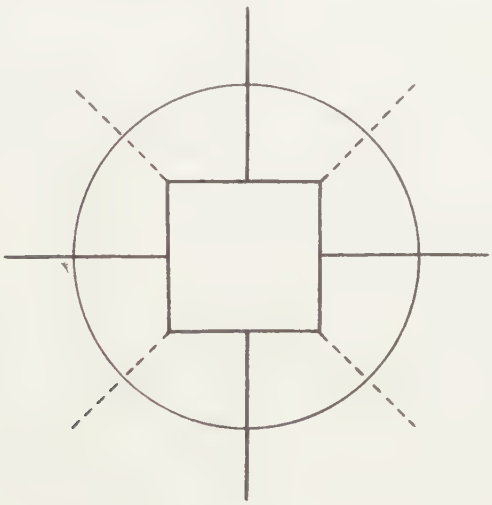


Fig. 2

Then this sphere can be considered as an isotropic crystal [2]) of the cubic class and the problem is thus reduced to that of inherent elastic anisotropy. The crystallographic axes of the imaginary crystal are directed at right angles to the faces of the cube. For a description of the elastic properties of the crystals it only is necessary to decide whether in the cross-section of fig. 2 the direction of the largest modulus is parallel to the sides of the square or to the diagonals. Only if loaded in the direction of the largest modulus, will no rotation occur, elastically and plastically. Loading in all other directions will result into a rotation, two-dimensionally of 45° maximally. If loaded in the direction of the smallest modulus, a trigger effect in the form of a small stress irregularity is needed to set in the rotation. The experiments, described in the next section, showed that the direction of the largest modulus lies diagonally.

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[1]) We referred to the influence of the form on pp. 124 and 127 (*l.c.*), but for the rest neglected it.

[2]) If taken two-dimensionally, the faces of the cube at the front and the back can be compared with etching figures.

If the cube is replaced by a rectangular parallelepiped, again an imaginary crystal is obtained, but now of the rhombic class. The direction of the largest modulus is easy to determine, as it represents the direction of the largest compressibility: at right angles to the largest face of the parallelepiped. In this case a rotation of 90° maximally may occur when the material starts flowing.

In a same way the influence of differently shaped inclusions can be evaluated.

Some experiments

A series of experiments was carried out, first to confirm the foregoing theoretical deductions, secondly to investigate the elastic properties of cubic inclusions. At first we tried to work with inclusions in modelling clay, which clay was compelled to flow out two-dimensionally. The results were not reliable, which probably must be ascribed to the circumstance that the apparatus was not constructed with enough precision to give a systematic, initial deformation. We therefore replaced the modelling clay by a very flexible rubber, which could be given elastic deformations such that they were no longer of the same order of magnitude as possible irregularities in the apparatus. Complications in the stress distribution due to small irregularities in the apparatus were nullified in this way. Instead of a finite plastic deformation governed by an infinite elastic deformation, we thus got finite elastic deformations. For our problem the difference is irrelevant. Inclusions were made from steel and from a less flexible rubber.

The following results were obtained. A cubic inclusion, placed under 20° with the direction of shortening (fig. 3a and b), rotated until its

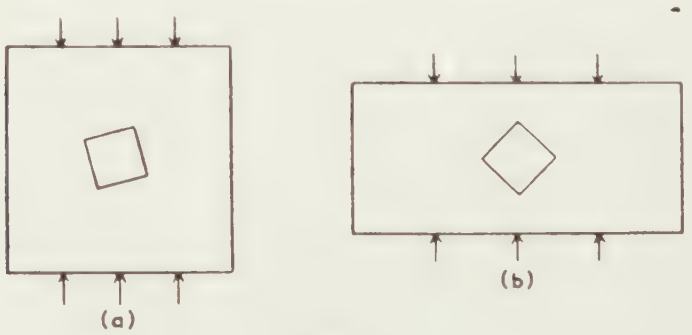

Fig. 3

diagonals were parallel to the directions of principal strain. To verify this outcome, inclusions were placed with their sides parallel to the axes of principal strain. Again rotation occurred, except in one case: here the inclusion obviously was at its dead point. Cubic inclusions placed with their diagonals parallel to the axes of principal strain, did not show any rotation.

This outcome decided the question in which direction the axes lie to which the largest modulus belongs: evidently the pseudo-crystal is more compressible in the diagonal directions. With a somewhat indirect reasoning the outcome may be made easy to understand (fig. 4). Suppose

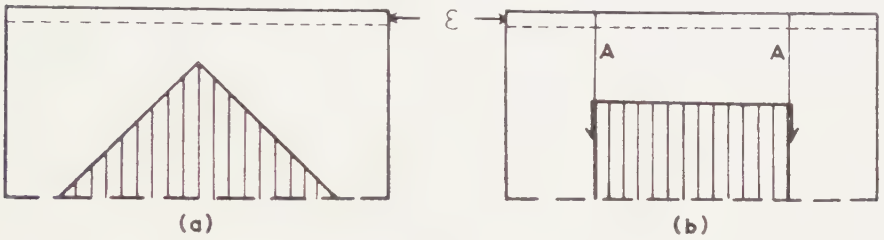

Fig. 4

the inclusion to be entirely rigid. The stresses in the material enclosing are more evenly distributed when the loading is parallel to the diagonals of the pseudo-crystal than when it is parallel to the sides of it: on the lines A a sudden transition occurs from a relatively low percentage of shortening at the outsides to a high one at the inside. Since this transition must be continuous, shearing stresses will occur which tend to bring down the corners of the inclusion. With regard to the center of inertia, these stresses form two couples which are in equilibrium as long as the geometry is perfectly symmetric. A small deviation, however, will destroy this equilibrium and a rotation will result. The equilibrium of fig. 4b is thus unstable, in contrast to the equilibrium of fig. 4a, which is stable.

Another experiment was made with an elongate inclusion of less flexible rubber (fig. 5). The rotation it showed was larger than was to be expected from the percentage of shortening.

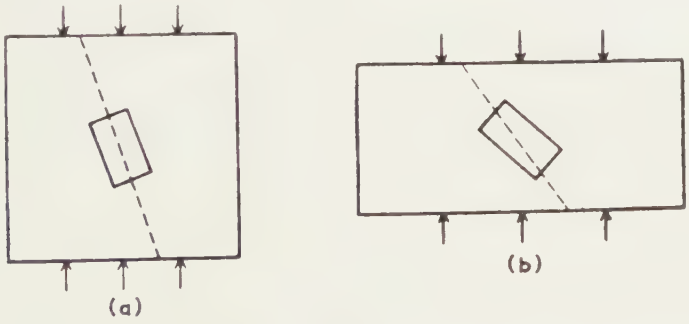

Fig. 5

In a way the experiments were already models of helicitic structures and they thus prove the validity of this way of theoretical approach.

Elastic anisotropy due to form: its effect upon crystal growth

It must be expected that the parallelism between mechanical rotation and orientated crystal growth, found to exist for inherent elastic anisotropy, will have its counterpart in the discussion of the elastic anisotropy related

to the form of an inclusion. With the principle of Riecke, elaborated by Ittmann and Rutten for the anisotropic case, the orientated growth of an crystal can be accounted for. Ittmann and Rutten showed that the strain energy, which lowers the equilibrium temperature of a two-phase system, depends on the orientation of the crystal. Hence only those crystals are stable, that lie with their axis to which the smaller of the elasticity constants belongs, in the direction of the maximum strain.

In order to arrive at an evaluation of the effect of the form of a crystal on the strain energy it takes up on recrystallizing under given strains, we consider a two-dimensional configuration as sketched in fig. 6, of unit

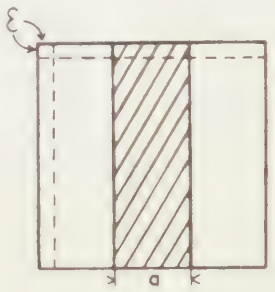

Fig. 6

cross-section. For convenience the harder inclusion of thickness a again is assumed to be isotropic. If a strain ε is given in the direction parallel to the longer side of the inclusion, the strain energy of the total system is

$$\tfrac{1}{2}a\varepsilon^2c' + \tfrac{1}{2}(1-a)\varepsilon^2c'' \text{ or } \tfrac{1}{2}a\varepsilon^2c'\left(1+\frac{1-a}{a}\cdot\frac{c''}{c'}\right),$$

c' being the elasticity constant of the inclusion, c'' that of the surrounding material. If, however, the strain is applied at right angles to the longer side of the inclusion, ε must be divided over the inclusion and the surrounding material in such a way that both materials produce the same stress σ. Writing $\sigma' = \varepsilon'c'$, $\sigma'' = \varepsilon''c''$ and $\varepsilon'c' = \varepsilon''c''$, σ' and ε' being the stress and the strain ruling in the inclusion and σ'' and ε'' the same quantities in the surrounding material, we obtain

$$\varepsilon' = \frac{\varepsilon}{a+(1-a)c'/c''}$$

and

$$\varepsilon'' = \frac{\varepsilon}{a\,c''/c'+(1-a)}.$$

The strain energy now must be written as

$$\tfrac{1}{2}a(\varepsilon')^2c' + \tfrac{1}{2}(1-a)\,(\varepsilon'')^2\,c''.$$

With $c' < c''$, we get $\varepsilon' > \varepsilon > \varepsilon''$, from which it follows that the strain energy of the inclusion is smaller and that of the surrounding material is larger than in the preceding case. The total strain energy is nevertheless smaller,

which can be seen by inserting the values of ε' and ε'' found above in the latter expression, which then can be written as

$$\tfrac{1}{2} a\varepsilon^2 c' \left\{ a^2 \left(1 + \frac{1-a}{a} \cdot \frac{c'}{c''} \right) \right\}^{-1}.$$

With regard to the expression for the first case the strain energy has thus been multiplied by

$$\{a^2 + (c'/c'' + c''/c') \cdot a(1-a) + (1-a)^2\}^{-1}$$

which factor is smaller than 1, $(c'/c'' + c''/c')$ always being larger than 2 [1]).

In our previous paper we arrived at the conclusion that with the strains given the orientation of minimum strain energy was the inverse to that with the stresses given. It was remarked that a situation in which the boundary conditions were expressed in stresses will seldom occur in nature. In the above given example it is even impossible to give the boundary conditions in the form of a stress parallel to the longer side of the inclusion. To produce the given stress the surrounding material would have to be strained more than the inclusion, which would involve a complication of the stress distribution and the origin of shearing stresses in the face normal to the direction of loading. We will not try to unravel these intermediary situations nor make an estimate of the strain energy taken up in those cases.

Since it can be supposed that a phenocrystal will always have higher elasticity constants than the surrounding polycrystalline material, it follows from the foregoing that if a crystal starts growing under given strains, it will tend to grow faster into the direction of the smallest strain, thus obtaining a form for which the strain energy, which is taken up instantaneously, is a minimum. If growing into a different direction, the phase equilibrium temperature is lowered and the crystal will go into solution again [2]). However, since there are also other factors affecting the form of a growing crystal, we can only speak of an influencing of the form. The ultimate form will represent the equilibrium of all factors involved.

The effect of the free surface energy on the form of a crystal

Another factor which is of importance for the equilibrium form of a crystal is the free surface energy (see e.g. HARTMAN and PERDOK, 1952). This energy term also influences the solubility of a crystal: the lower this energy, the less soluble the crystal. In an entirely homogeneous solution, i.e. a solution in which no temperature or concentration gradients exist,

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[1]) If we assume that there is an energy relation between the inclusion and the surrounding material, we again obtain a model of an anisotropic crystal, the inclusion representing a more compact layering. c_{11} in a direction parallel to the longer side of the inclusion then is the larger constant.

[2]) GRUBENMANN–NIGGLI (1924, p. 464) already formulated a similar rule: the strongest solubility is in the direction of the largest strain.

the growth of those crystals will be favoured that have a as small surface of high energy faces as possible.

For a crystal growing under a given strain the combined energy effect must form a minimum. It is therefore conceivable that a crystal under a certain strain grows faster into the direction of maximum strain than in the other directions, the free surface energy term being higher than the strain energy term requiring this. When the strains increase this form will no longer be stable and recrystallization may take place by which the form of the crystal is flattened in the direction of the maximum strain. But also mechanical reorientation may occur, on the lines dealt with above.

Schistosity

In our paper on schistosity only the effect of inherent elastic anisotropy was considered. Considerations on the effect of the form of the mica flakes on their orientation lead to the same result: evidently also the strain energy term related to the form of the crystal reaches its minimum if the flakes are orientated at right angles to the direction of maximum strain. It is difficult to say which of the two factors is the more important one, but this is of no relevance to the orientation problem dealt with.

Since mica can be considered to be hexagonal up to a high degree, the energy term related to the inherent elastic anisotropy cannot contribute to the explanation of the occurrence of elongate crystals in the tectonical *b*-direction. But the strain energy term related to the form of the flakes is susceptibel to strain differences at right angles to the direction of maximum strain. In a two-dimensional problem (and many geological structures may be considered to be in principle two-dimensional) the strain in the direction of the axis of no deformation (the tectonical *b*-axis) must be put zero. The strain energy term will thus reach a minimum if the longer axis of an inclusion lies into this direction, i.e. the formation of elongate crystals with their longer axes into the *b*-direction will be favoured. In the case of mica the free surface energy term will favour the growth of real flakes, if possible combined into larger plates. It now may be assumed that this tendency is the stronger one till a critical strain difference is reached in the plane at right angles to the maximum strain. If the strain difference is higher, elongate mica crystals will appear (fig. 1).

The occurrence of elongate crystals in the direction of the folding axis

The foregoing reasoning can readily be applied to other crystals. Generally the inherent anisotropy will not be as large as for mica, but the question of the form of the crystal, and of the free surface energy term remains important. This means that phenocrystals growing under critical strain conditions will tend to develop farther into the tectonical *b*-direction, than in the other directions (see fig. 1). The crystallographic axes may be orientated according to the axes of the strain ellipsoid: this depends on the circumstance whether the effect of inherent anisotropy is larger than

the irregularities of form which a crystal even in its first stage of growth will show.

If the differential strain is not large, the free surface energy term may become the more important one. In that case a haphazard orientation may result, or, if the crystallographic axes were orientated, an orientation of the longer axes in a direction different from the tectonical *b*-direction.

Helicitic structures

If a situation as referred to in the preceding section occurs (an orientation into a direction other than that of the *b*-axis, or a haphazard orientation), and if afterwards the strains increase and the material starts flowing, then the phenocrystals will start rotating. The same will happen if the free surface energy term caused the crystal to adopt a cubic shape with the face normals parallel to the axes of the strain ellipsoid. As became clear from the experiments this is not a stable configuration, the strain energy term related to the form being maximal. The resulting rotation can be maximally 90° for an elongate phenocrystal (i.e. until its longer axis coincides with the direction of minimal strain) and 45° for a cubic one. Of course these maxima can only be reached if there is sufficient plastic shortening.

When the crystal growth continues, this growth may still be into the direction of maximum strain if the free surface energy term is large enough [1]). Thus the spiralshaped structures originate. If the growth extends into one direction only, a single spiral results, with growth into two opposite directions a double spiral. As long as the growth continues no ultimate equilibrium position is reached and this explains why rotations of 360° and more can occur.

Also phenocrystals, such as consisting of garnet, having the shape of a highly developed polyhedron may give birth to helicitic structures, since a small irregularity in the crystal growth is able to destroy the geometric symmetry. Mechanically the phenocrystal is then reduced to a lower symmetry class.

The schistosity planes become curved and contorted by the rotation. In the strain shadow beside the crystal new minerals may originate or mica may recrystallize. Thus the total aspect of helicitic para- or post-crystalline structures comes into being.

A field observation

In an augengneiss in the Ötz valley near Köfels (Austria) we observed the following phenomena. Phenocrystals of potash felspar, of rectangular cross-section, presumably grown with the longer sides of the rectangle at right angles to the schistosity plane, had been rotated from that

[1]) Continued growth into the direction of maximum strain might, of course, also be a question of supply of material, i.e. concentration gradients.

position over an angle of about 30°–40°. The deformation was clearly postcrystalline. As a rule the sense of rotation in one layer was the same for all crystals. In one instance, however, the sense of rotation changed abruptly. Furthermore, it was not always the same for two different layers, but it changed haphazardly at right angles to the schistosity plane.[1])

In this case we clearly have to do with "active" rotation of the crystals, as described above. Generally, the way one crystal sets to rotate will determine the sense of rotation of its close neighbours, i.e. those in the same "layer". Exceptions are conceivable since two crystals may start to rotate at exactly the same moment in an opposite sense. There is no reason, however, why a rotating crystal should influence the sense of rotation of crystals in the neighbouring layers and this explains the haphazardness of rotational sense over a greater rock surface.

Conclusions

The theory of elastic anisotropy, recently used by the author in the explanation of the origin of schistosity but at that moment applied only to inherent elastic anisotropy, appeared also to be applicable to inclusions in an isotropic medium showing an anisotropy of form. Again a mechanical effect (reorientation by active rotation of the inclusion) and a physico-chemical effect (orientated recrystallization) can be discerned. In recrystallization questions also the free surface energy may play an important role.

The theory explains a series of geological phenomena as the occurrence of schistosity, of a second schistosity (which may be accompanied by recrystallization), of elongate crystals in the direction of the axis of a fold and of helicitic structures of the para- and postcrystalline deformation type. All these phenomena can be related to an elastic strain condition in which the maximum strain was directed at right angles to the schistosity plane, in the case of a second schistosity plane at right angles to that plane. These strains have become petrified by plastic flow and recrystallization.

With regard to the origin of helicitic structures, this implies that no external rotation has occurred, and *mutatis mutandis*, that these structures cannot be considered as witnesses of shearing movements parallel to the schistosity plane, nor can be used to evaluate the amount of such a shearing. Some field observations confirm this outcome.

[1]) We observed similar phenomena in the Gamsbodengneiss of the Gotthard Massif.

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PLANKTONIC FORAMINIFERA IN THE OLIGOCENE AND MIOCENE OF THE NORTH SEA BASIN

BY

C. W. DROOGER AND D. A. J. BATJES

(Communicated by Prof. G. H. R. VON KOENIGSWALD at the meeting of May 30, 1959)

ABSTRACT The planktonic associations of the Oligocene sediments of the North Sea basin consist of but a few, small, intergrading *Globigerina* types. Those of the Miocene deposits are often more varied and then characterized by considerable numbers of *Globigerinoides triloba*, *Globigerina* cf. *nepenthes*, *G. ciperoensis* and *Globorotalia* cf. *scitula*.

INTRODUCTION

The detailed stratigraphy of Tertiary sediments of tropical and subtropical regions is more and more being based on planktonic Foraminifera. Several species and variants in this group are morphologically well recognizable, and some of them appeared to be good index fossils for long distance correlations. Especially during the last decade, the investigations have led to the establishing of the ranges of many types, as for instance in the Oligocene and Miocene deposits of Trinidad. Whether these types are all good guide fossils for interregional parallelizations has still to be checked.

Planktonic Foraminifera of Tertiary sediments of the North Sea basin have never yet been studied in detail, probably because of the rare and poor associations of usually small and rather uncharacteristic individuals. Our preliminary results deal with Oligocene and Miocene forms, found in numerous samples of outcrops in Belgium and Germany, and in samples of some of the older borings in the Peel region of the southern Netherlands (Maasbree-13, Oplo-16, Swalmen-21), and of some subsurface sections in western Germany, the most important of which is that of the mine shaft Kapellen-3.

Very few references to planktonic species are to be found in the literature on the Foraminifera of the Oligocene and Miocene of the North Sea basin. Since ROEMER in 1838, who already recognized three different types in the Osnabrück deposits, there has been no real advance. Authors (REUSS, HOSIUS, STAESCHE and HILTERMANN, TEN DAM and REINHOLD, etc.) all determined their planktonic species with one or a few of the more general names, and many of these determinations are incorrect.

For our determinations the recent paper of BOLLI (1957) was taken as an important reference, mainly because of the thorough splitting of

the assemblages into types with known stratigraphic ranges in the Oligocene and Miocene sediments of Trinidad. Many of these types certainly do not represent biological species, but as yet this excessive splitting is considered acceptable as the easiest means of expressing the observed variation. A series of samples from Trinidad, kindly offered by Dr. H. M. Bolli, was available for comparison.

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Dr. A. A. Thiadens, director of the Netherlands Geological Survey (Haarlem), who kindly authorized the publication of data from the older borings of the Rijksopsporing van Delfstoffen in the Peel region,

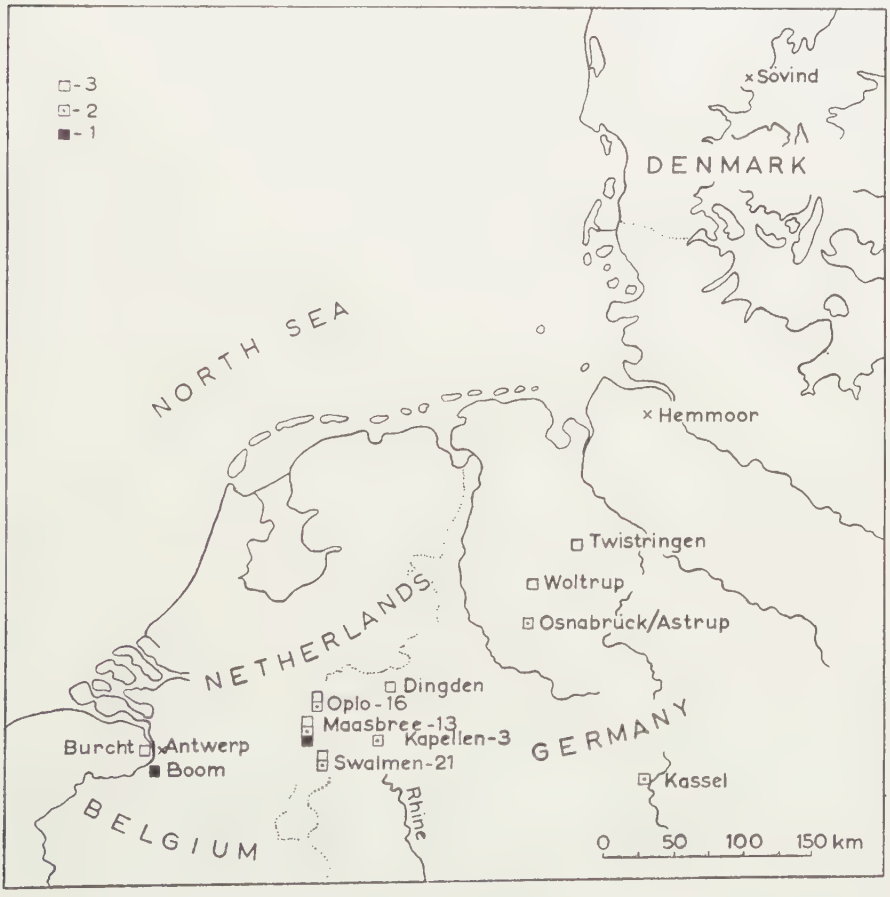

Fig. 1. Localities mentioned in the text. 1 = Rupel clay. 2 = *Asterigerina beds*, *Elphidium-Almaena* beds. 3 = Hemmoor beds, Dingden beds, Antwerp sands, etc. (Miocene).

The Netherlands Organization of Pure Scientific Research, (The Hague), for the financial support which enabled a field trip in Germany in 1956, and the Bundesanstalt für Bodenforschung, (Hannover), for the ample assistance during this trip,

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SYSTEMATIC DESCRIPTION

Part of our determinations are tentative. When very small individuals have been given the name of bigger types, as for instance in the case of *Globigerina bulloides*, this has not been expressed in the determination.

Globigerina ampliapertura Bolli

Pl. 1, fig. 1

Globigerina ampliapertura Bolli, 1957, U. S. Nat. Mus., Bull. 215, p. 108, pl. 22, fig. 4–7.

Globigerina globularis Roemer, Drooger, 1956, Micropal., vol. 2, p. 184, pl. 1, fig. 1, 2 (not fig. 7, 8, 17).

This *G. ampliapertura* is a fairly big, high-chambered type of Upper Eocene and Oligocene deposits of the Tethys. For Europe it was figured from the Oligocene of Biarritz (Drooger). Since the size of the Oligocene globigerinids in our material of the North Sea basin is small to moderate, this may be a reason for the general absence of *G. ampliapertura* in this region. Thus far we only have big individuals of this type from a sample of the Danish Sövind marl of probably Eocene or Early Oligocene age (Sorgenfrei, 1957).

Globigerina globularis Roemer

Pl. 1, fig. 2

Globigerina globularis Roemer, 1838, N. Jahrb. Min. Geogn. Geol. Petr. k., p. 390, pl. 3, fig. 57; Drooger, 1956, Micropal., vol. 2, p. 184, pl. 1, fig. 7, 8, 17 (not fig. 1, 2); Batjes, 1958, Verh. Kon. Belg. Inst. Natuurw., no. 143, p. 161, pl. 11, fig. 3–5.

Globigerina cf. *trilocularis* d'Orbigny, Bolli, 1957, U. S. Nat. Mus., Bull. 215, p. 110, pl. 22, fig. 8, 9.

In the Trinidad material specimens identical with Bolli's *G.* cf. *trilocularis* are of fair size. They are mostly smaller variants of the connected *G. ampliapertura* type. They are different from the latter in the less rapid increase in height of the later chambers. The dorsal face is more or less flattened; there are 3 to 3½ chambers in the final coil. The type somewhat resembles those of *Globigerinoides triloba triloba* and *G. triloba immatura*, but it differs by the lack of dorsal openings. It was thought by Bolli to be the ancestor of these *Globigerinoides*.

The type of *Globigerina globularis* is not very clear. Roemer's original

description contains but few details: "Besteht aus 3–4 mit einander etwas verschmolzenen Kammern". The localities he gave are Kassel and Osnabrück (= Astrup). In our material of the "Meeressand" of Kassel distinct autochthonous Globigerinidae are lacking. In 1956 we figured a specimen from Astrup (DROOGER, fig. 8), but such individuals with 3 to $3\frac{1}{2}$ chambers in the final coil are not frequent in our material from that locality. They were found to grade into the far more common four-chambered specimens, which we determine as *G.* cf. *suteri* (see below). However, ROEMER's figures suggest a three-chambered type, whereas our *G.* cf. *suteri* is clearly identical with ROEMER's *G. trilocularis* type.

ROEMER credited *G. globularis* to D'ORBIGNY (1826), but ROEMER's are the first description and figures of a *Globigerina* species under this name. FORNASINI's description of D'ORBIGNY's material in 1904 introduced a late homonym. Moreover, ROEMER's species has no relation to that of D'ORBIGNY.

No doubt our present interpretation of *G. globularis* is still very wide. The biggest specimens in our material (up to 0.40 mm) are those from Astrup and corresponding assemblages of the *Elphidium-Almaena* beds of the Peel borings. But small *G. globularis* individuals (up to 0.35 mm) are among the most common types of the Rupel Clay assemblages (DROOGER, fig. 7; BATJES, fig. 3–5) and they also occur among the very small individuals of the overlying *Asterigerina* beds, both of Oligocene age. Small individuals of again the same type were encountered in many of the samples of the Miocene. These Miocene types are possibly identical with those from the Burdigalian of the Aquitaine basin (DROOGER, fig. 17). They are probably small variants of *Globigerinoides triloba* and *Globigerina* cf. *nepenthes*.

Another type, regularly met with, especially in our Oligocene material, is characterized by an aberrant final chamber. It most closely resembles *G. unicava* (BOLLI, LOEBLICH and TAPPAN) (*Catapsydrax unicavus* BOLLI, LOEBLICH and TAPPAN, 1957, U. S. Nat. Mus., Bull. 215, p. 37, pl. 7, fig. 9). The individuals are clearly related with our *G. globularis* group.

Globigerina bulloides D'ORBIGNY

pl. 1, fig. 3

Globigerina bulloides D'ORBIGNY, 1826, Ann. Sci. Nat. ,ser. 1, vol. 7, p. 277, mod. 17, 76; CUSHMAN, 1914, Contr. Cushm. Lab. Foram. Res., vol. 17, p. 38, pl. 10, fig. 1–13; BATJES, 1958, Verh. Kon. Belg. Inst. Natuurw., no. 143, p. 161, pl. 11, fig. 1, 2.

This general type occurs in small numbers throughout our Oligocene-Miocene sequence. The individuals are nearly always smaller than the typical (see CUSHMAN); they are variously connected with other types, and they are most frequent in the Oligocene deposits.

Globigerina parva BOLLI

Pl. 1, fig. 4

Globigerina parva BOLLI, 1957, U. S. Nat. Mus., Bull. 215, p. 108, pl. 22, fig. 14.

Globigerina cf. *bulloides* d'Orbigny, Drooger, 1956, Micropal., vol. 2, p. 184, pl. 1, fig. 6.

Bolli established this species for small four-chambered specimens of his *ampliapertura*-zone (Oligocene). Their oblique aperture is confined to the final chamber and not externally connected with the apertures of earlier chambers.

In our material the *G. parva* individuals grade into the *bulloides*, *globularis* and *angustiumbilicata* types. They too, occur mainly among the small individuals of the various Rupel clay camples.

Globigerina angustiumbilicata Bolli

Pl. 1, fig. 5

Globigerina ciperoensis angustiumbilicata Bolli, 1957, U. S. Nat. Mus., Bull. 215, p. 109, pl. 22, fig. 12, 13.

Globigerina cf. *increbescens* Bandy, Drooger, 1956, Micropal., vol. 2, p. 184, pl. 1, fig. 4, 5.

Globigerina sp. Batjes, 1958, Verh. Kon. Belg. Inst. Natuurw., no. 143, p. 161, pl. 11, fig. 7, 8.

This form was described by Bolli from Trinidad as a variant of his *G. ciperoensis*, with which it occurs together in the Oligocene. However, in this island it outlives *G. ciperoensis* s. str., ranging up to the *dissimilis*-zone.

Also in our material this type was found together with *G. ciperoensis*, but here in Miocene deposits. Similar small specimens (up to 0.4 mm) occur in samples from the Oligocene, without distinct *G. ciperoensis*. They are clearly connected with the other types, already described. They were figured under different names from the Oligocene Rupel clay.

Globigerina cf. suteri (Bolli)

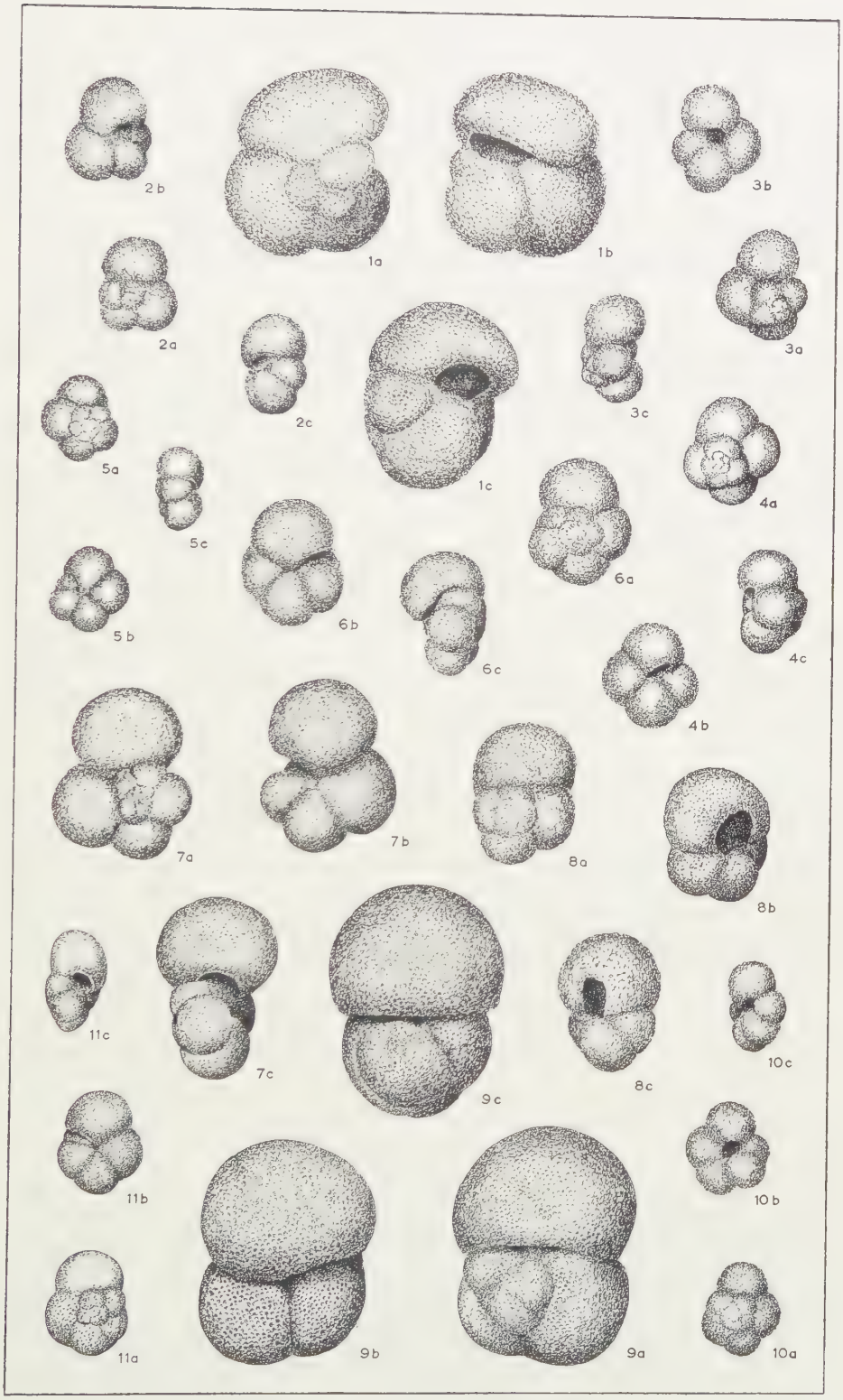
Pl. 1, fig. 6

Globigerina trilocularis, Roemer (not d'Obigny), 1838, N. Jahrb. Min. Geogn. Geol. Petr. k., p. 390, pl. 3, fig. 41*a*.

Plate 1. All figures ×60. *a* = dorsal view, *b* = ventral view, *c* = peripheral view.

1. *Globigerina ampliapertura* Bolli. Sövind marl, Sövind-3, Denmark.
2. *Globigerina globularis* Roemer. *Elphidium-Almaena* beds, boring Swalmen-21, 220 m, Netherlands.
3. *Globigerina bulloides* d'Orbigny. *Elphidium-Almaena* beds, boring Swalmen-21, 225 m, Netherlands.
4. *Globigerina parva* Bolli. Rupel clay, boring Maasbree-13, 476 m, Netherlands.
5. *Globigerina angustiumbilicata* Bolli. Rupel clay, MA 658, Boom, Belgium.
6. *Globigerina* cf. *suteri* (Bolli). *Elphidium-Almaena* beds, BA 7, Astrup Germany.
7. *Globigerina obesa* (Bolli). Miocene, TS 4, Twistringen, Germany.
8. *Globigerina* cf. *nepenthes* Todd. Miocene, boring Swalmen-21, 81 m, Netherlands.
9. *Globigerinoides triloba* (Reuss). Miocene, TS 1, Twistringen, Germany.
10. *Globigerina ciperoensis* Bolli. Miocene, Burcht near Antwerp, Belgium.
11. *Globorotalia* cf. *scitula* (Brady). Miocene, boring Oplo-16, 152 m, Netherlands,

PLATE 1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Cf. *Globorotaloides suteri* Bolli, 1957, U. S. Nat. Mus., Bull. 215, p. 117, pl. 27, fig. 9–13.

In our material *G.* cf. *suteri* is certainly not an independent type; it represents a rather variable group of individuals. It is most numerous in some associations of the *Elphidium-Almaena* beds of the borings Oplo and Swalmen, and it also occurs in considerable numbers in our samples from Astrup.

Roemer's species from Osnabrück (= Astrup) must be identical with ours. His figure is fairly distinct, especially when it is combined with his description: "Besteht aus 3–4 etwas niedergedrückten Kammern".

The individuals have four, and sometimes up to five chambers in the final coil. They are of intermediate size (up to 0.45 mm), with flattened dorsal side and an aperture of variable height, which extends from the umbilicus to a point close to the periphery. The wall is usually coarsely perforated and rugose. Fairly thick-walled specimens with flat dorsal side, coarse surface and a low aperture with a slight lip, are considered to be the most characteristic.

Our specimens are often the four-chambered equivalents of *G. globularis*, in which the chambers do not increase so rapidly in size. Individuals with looser coiling resemble *G. obesa*. The limit with *G. angustiumbilicata* is also not clear. Especially in bigger specimens the last chamber may have been added more ventrally, the test thus becoming dorsally more convex, and the aperture usually being higher. This type is frequent among our Astrup individuals. The lips in the aperture sometimes suggest *Globoquadrina* features.

In the Trinidad samples we found very similar types, especially in the samples of the *ampliapertura* and *ciperoensis*-zones. In our opinion there is the same relation with the other types as there is in our material from the North Sea basin. According to Bolli the vertical range of *G. suteri* is from *ampliapertura to insueta*-zone, but he also figured this species from the Eocene Navet formation of Trinidad (1957, p. 166, pl. 37, fig. 10–12).

Probably our group has a wider variation than Bolli meant it to be for his species. For instance, most of our individuals lack the lip of the aperture, but this we also observed in many of our specimens from Trinidad.

Globigerina obesa (Bolli)

Pl. 1, fig. 7

Globorotalia obesa Bolli, 1957, U. S. Nat. Mus., Bull. 215, p. 119, pl. 29, fig. 2, 3.

Fairly big specimens (up to 0.6 mm) from the higher, Miocene parts of the sections, closely resemble the Trinidad individuals of this species. They are probably related to *G. concinna* Reuss as it was found in the Helvetian and Tortonian deposits of the Vienna basin (Drooger, 1956, pl. 1, fig. 33, 34). The most common types of the latter species, with flattened dorsal sides, only differ from our *G. obesa* individuals in the

higher number of chambers in the final convolution. They occur seldom among our individuals of the North Sea basin.

Similar, but smaller individuals of this *obesa* type occur in the *Elphidium-Almaena* beds, in which they were found to intergrade with *C.* cf. *suteri*.

In Trinidad, *G. obesa* has a stratigraphic range from the *dissimilis* up to the *menardii*-zone.

Globigerina ciperoensis Bolli

Pl. 1, fig. 10

Globigerina ciperoensis Bolli, 1954, Contr. Cushm. Found. Foram. Res., vol. 5, p. 1, textfig. 3–6; Batjes, 1958, Verh. Kon. Belg. Inst. Natuurw., no. 143, p. 162.
Globigerina ciperoensis ciperoensis Bolli, Bolli, 1957, U. S. Mus., Nat. Bull. 215, p. 109, pl. 22, fig. 10.

We failed to find any notable morphologic difference between our numerous individuals and those of our Trinidad material. For instance, they are identical in size (up to 0.35 mm), in the trochoid coil of $4\frac{1}{2}$ to 5 chambers around an open umbilicus, and in the finely hispid character of the wall. Occasionally there is intergradation with *G. angustiumbilicata*, but *G. ciperoensis* var. *angulisuturalis* Bolli (1957, p. 109, pl. 22, fig. 11) was not observed. There is no clear connection of our specimens with bigger *Globigerina* types. They are distinct and frequent only in the higher, Miocene part of the sections, such as in the samples from the Antwerp sands.

In Trinidad this species is restricted to the Oligocene *ampliapertura* to *ciperoensis*-zones. An Oligocene age would be unacceptable for the North Sea deposits with *G. ciperoensis*, not in the least because of the accompanying planktonic types. Either we are dealing with the survival of this species in a remote area, or with an example of later variation, which included a type that is a copy of an earlier form. Such variation may be expected among the Globigerinidae with their relatively simple morphology.

Globigerina cf. nepenthes Todd

Pl. 1, fig. 8

Cf. *Globigerina nepenthes* Todd, 1957, U. S. Geol. Survey, Prof. Paper 280-H, p. 301, pl. 78, fig. 7; Bolli, 1957, U. S. Nat. Mus., Bull. 215, p. 111, pl. 24, fig. 2.

The dorsal side of our specimens is usually rather flat. The chambers increase rapidly in size; there are $3\frac{1}{2}$ to 4 in the final coil. Especially in four-chambered types the dorsal side is somewhat more convex, because the last chamber has been added more obliquely in ventral direction. The wall is fairly thick, coarsely cancellated to rugose. Characteristic is the very wide and high aperture with smooth rim, mainly lying between umbilicus and periphery. The diameter of the test amounts to up to 0.45 mm.

Smaller individuals in some of the samples grade into those of the

G. globularis type, which is different by the much lower aperture. Among the four-chambered variants there is occasionally transition to the *G. bulloides* type, which has the aperture more umbilical and opening into all chambers, ventrally visible.

G. cf. *nepenthes* was only found in Miocene sediments of the North Sea basin. Biologically it is considered to be a variant of *Globigerinoides triloba*. In our material both types have similar coarse walls. The loss of the dorsal apertures of *G. triloba* is thought to be compensated by the much larger final aperture of *G.* cf. *nepenthes*. The *G.* cf. *nepenthes* specimens are always smaller than the majority of those of *G. triloba*. Usually both types do not occur together in one sample. In the boring Swalmen they were found to alternate in successive samples. Possibly both forms were "ecophenotypes" of one species, restricted to their own "ecologic water masses" (PHLEGER, 1954), the characteristics of which are unknown.

For comparison individuals of *G. nepenthes* from the *mayeri* and *menardii*-zones of Trinidad were available. They closely resemble BOLLI's figures. They are different from our specimens by the more compact four-chambered form with less increase in chamber size, by the somewhat pointed character and lesser size of the final chamber, and by the smaller aperture. *G. druryi* AKERS (1955, Jour. Pal., vol. 29, p. 654, pl. 65, fig. 1) from the Miocene of Louisiana, seems to be very close to, or identical with *G. nepenthes*; possibly it is an even smaller variant. In our opinion there is little doubt that these American forms also belong to the *G. triloba* group. They seem to be small, thin-walled variants, also related to early *Sphaeroidinella* (*S. grimsdalei* (KEYZER)). In our Trinidad material, individuals of this group with chambers more rapidly increasing in size, have a dorsal aperture and the final chambers are flattened. These specimens resemble *Globigerinoides obliqua* BOLLI.

As yet our *G.* cf. *nepenthes* is of little value in correlation of the North Sea deposits with those of the Tethys. There is no reason to parallelize them with the two upper (*mayeri* and *menardii*) zones of Trinidad, which are the only zones with *G. nepenthes*. Specimens, similar to those of the North Sea basin, though smaller, were encountered in the material of the *dissimilis*-zone of Trinidad, the lowest zone with *G. triloba*. They resemble our *G.* cf. *nepenthes* of the North Sea basin better than *G. nepenthes* of both upper Trinidad zones. However, such types may be expected to occur at all levels with *G. triloba*.

Globigerinoides triloba (REUSS)

Pl. 1, fig. 9

Globigerina triloba REUSS, 1850, Denkschr. Ak. Wiss. Wien, Math.-Nat. Kl., vol. 1, p. 374, pl. 47, fig. 11; TEN DAM and REINHOLD, 1942, Meded. Geol. St., ser. C-V, no. 2, p. 96, pl. 7, fig. 8.

Globigerinoides triloba (Reuss), DROOGER, 1956, Micropal., vol. 2, p. 185, pl. 1, fig. 18, 36; BOLLI, 1957, U. S. Nat. Mus., Bull. 215, p. 112, pl. 25, fig. 2, textfig. 21–1.

Our specimens are usually of considerable size, up to 0.65 mm. Most of them show the rapid increase in size of the chambers and the low apertures of Bolli's *G. triloba triloba*. Part of the individuals resemble *G. triloba immatura* Le Roy (see Bolli, pl. 25, fig. 3, 4; textfig. 21–2).

All occurrences of the North Sea basin are in the Miocene. In Trinidad *G. triloba* – and the genus *Globigerinoides* – first appear in the *dissimilis*-zone. In the Mediterranean area this beginning was in the Aquitanian-Burdigalian time interval.

Globorotalia cf. **scitula** (Brady)

Pl. 1, fig. 11

Cf. *Pulvinulina scitula* Brady, 1882, Proc. R. Soc. Edinb., vol. 11, p. 716. *Pulvinulina patagonica* Brady, 1884, Rep. Voy. Challenger, Zoology, vol. 9, pl. 103, fig. 7.

Globorotalia scitula (Brady), Bolli, 1957, U. S. Nat. Mus., Bull. 215, p. 120, pl. 29, fig. 11, 12.

Globorotalia scitula Vašíček (not *Pulvinulina scitula* Brady), 1951, Geol. Surv. Czechosl., vol. 18, Paleont., p. 180, pl. 2, fig. 14, textfig. 7.

Our specimens resemble *G. scitula* in the low number of chambers, which is mostly four, but may be as many as five. They are different by the more rounded periphery. Furthermore, the ventral side is much more convex than the dorsal side, which is mostly nearly flat. The test is higher than in typical *G. scitula*. Coiling is without preference of direction. The dorsal sutures are but slightly curved. The wall is coarsely perforated, especially in the earlier chambers, which are also more rugose and often conspicuously cancellated. There is a wide aperture, extending from the umbilicus about two thirds of the distance to the periphery. The diameter of the test is up to 0.35 mm.

G. cf. *scitula* is another species, which in the North Sea basin is restricted to some samples of the Miocene deposits.

The specimens described and figured by Vašíček from Moravian Miocene sediments (Tortonian) seem to be closest to ours. They resemble our individuals in the general shape of the test, but there are five chambers in the final coil of the figured specimens, and the wall possibly is less rugose. Although figured, the planoconvex shape of the test does not seem to be the rule, as it is in our material.

Our specimens also show resemblance with *Globigerina mayeri* (Cushman and Ellisor) and *Globorotalia fohsi barisanensis* Le Roy, as figured by Bolli (1957, pl. 28, fig. 4 and 8, respectively), but both have more chambers in the final coil. A similar individual was figured by Drooger from the Tortonian of the Vienna basin (1956, pl. 1, fig. 31). His *G. canariensis* (d'Orbigny) (id., fig. 37) from the Tortonian of Tortona closely resembles *G. scitula* of both Brady and Bolli.

For correlation with Trinidad our *G.* cf. *scitula* seems to be of little use. There are some "primitive" *Globigerina* features, as for instance in the

wall structure, which suggest a position close to the origin of the Miocene *Globorotalia*. The first real Miocene *Globorotalia* species in the Trinidad section, *G. fohsi barisanensis*, ranges from the *dissimilis* to the *fohsi barisanensis*- zone. This would fit in better with the ranges of the other North Sea species than the vertical distribution of *G. scitula* in Trinidad (*fohsi fohsi* to *menardii*-zone). *Globigerina mayeri* is of little use; its range is throughout nearly the entire Oligo-Miocene section of Trinidad.

The primitive *Globorotalia* types of Moravia and the Vienna basin occur in sediments of Tortonian age (with *Orbulina*), but they were not found in the Helvetian deposits. The age determinations of these sediments are thought to be more reliable in this Central European area than elsewhere, since in the same sedimentary through we find the type deposits of the Helvetian (Papp, 1958).

STRATIGRAPHIC CONCLUSIONS

As might be expected our planktonic assemblages cannot nicely be fitted in with the Trinidad section of Bolli (fig. 2). They often consist of very small to intermediate sized individuals, which are hard to compare with the bigger types of the Tethys. This small size may partly be due to sorting, but a relatively low temperature of the water may have been equally important. No doubt climatic factors influenced the character of the associations, just as they are found to do in the present oceans from low to high latitudes, not only influencing the size of the individuals but also their morphologic features. In this way they may be responsible for the approximative determinations of several of our species.

As a whole our present knowledge of the planktonic Foraminifera of the North Sea basin enables us to distinguish only two different and successive groups of associations. In the literature the deposits in which they were found, are generally referred to as Oligocene and Miocene. There are indications that a further subdivision may be possible, but many more data from continuous sections have to be gathered.

In the Rupel clay of Belgium, Germany and the Netherlands there is a monotonous fauna of small intergrading *Globigerina* types: *G. globularis*, *G. bulloides*, *G. parva*, *G. angustiumbilicata*. Bigger types, among which *G. ampliapertura*, were thus far only found in the Danish Sövind marl, which is of Early Oligocene or Eocene age (Sorgenfrei, 1957).

The overlying *Asterigerina* beds of Germany and the Netherlands (Staesche and Hiltermann, 1940; Ten Dam and Reinhold, 1942) contain hardly any planktonic Foraminifera, as may be seen from the tables given by Indans (1958) and Ellermann (1958).

The next higher rock stratigraphic unit, that of our *Elphidium-Almaena* beds [1]) (unit 5 of Ellermann, 1958; unit F of Indans, 1958) with its

[1]) After *Elphidium submodosum* (Roemer) and *Almaena osnabrugensis* (Roemer). The type locality is Astrup near Belm, 8 km ENE of Osnabrück.

rich benthonic microfauna (BATJES, 1958), was evidently deposited under conditions more favorable to planktonic life. In part of our samples (Astrup, Swalmen 225–122 m, Oplo 176–154 m), the individuals are often of more intermediate size. This greater size may be the reason that more types seem to be present than there are in the two underlying rock units. The most important of these additional types is *Globigerina* cf. *suteri*.

In the *Elphidium-Almaena* beds we found smaller globigerinids in the mine shaft Kapellen-3 than in the Peel borings. These beds are furthermore remarkable because of their small, many-chambered *Globigerina* and equally small *Gumbelina* specimens. These individuals of several species have probably been derived from Upper Cretaceous and Lower Tertiary strata. Such elements were not found in Astrup, they are scarce in the Peel, constitute about fifty per cent of the planktonic individuals at Kapellen, and are the only, common planktonics in our samples of the "Meeressand" of Kassel. Although these *Elphidium-Almaena* beds need not be strictly synchronous at all these places, we may obtain some idea from this distribution about the direction of transport of the fine clastic material.

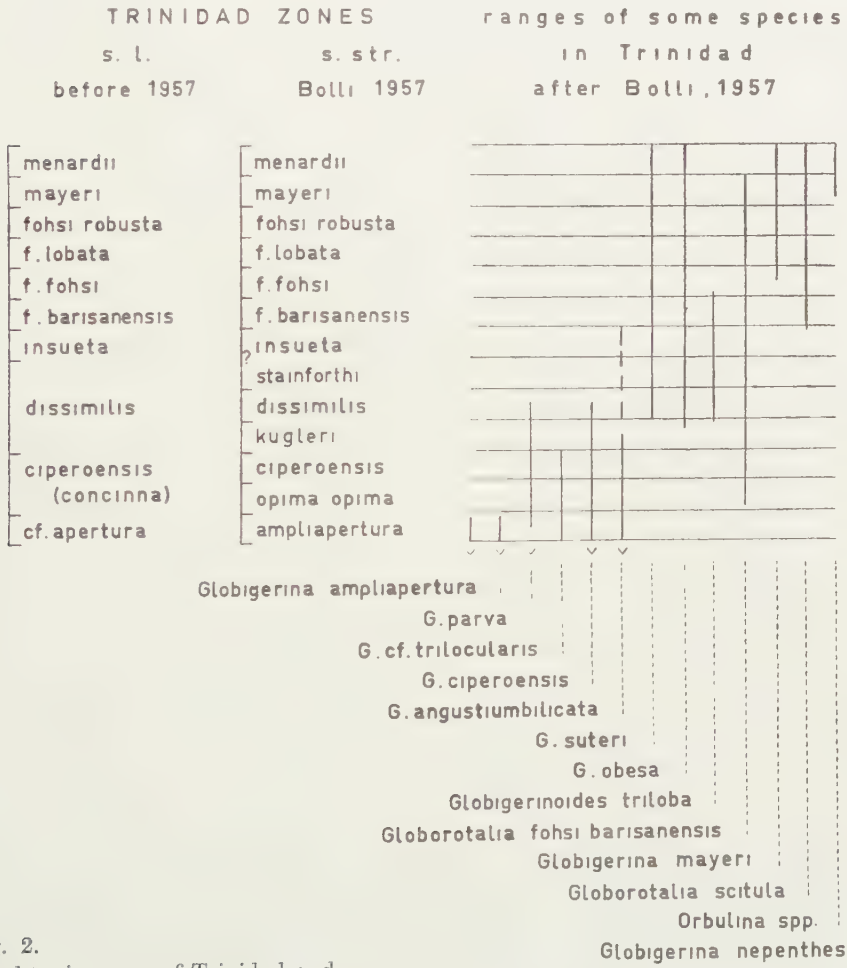

Fig. 2.
Planktonic zones of Trinidad and ranges of some of the species. V = also reported from Eocene deposits of Trinidad.

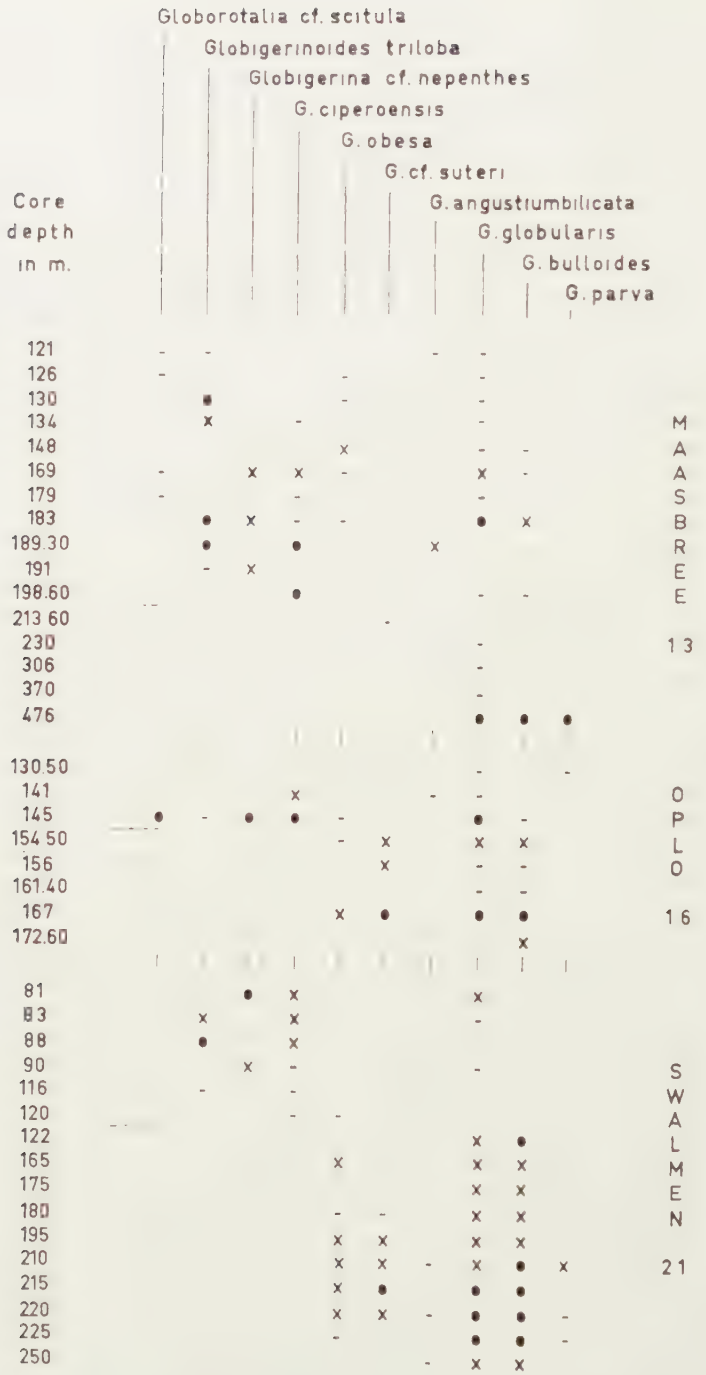

Fig. 3. The planktonic types in the Oligocene-Miocene of three borings of the Peel region. The vertical arrangement is not to scale. In the samples in between there are no or only very few planktonic specimens. — = 1 or 2 individuals, × = 3–10 individuals, ● = more than 10 individuals. These numbers can be used for a qualitative analysis only. Planktonic individuals being scarce in nearly all samples, they were picked without system, on various occasions.

The autochthonous associations, discussed so far, all consist of a small number of intergrading *Globigerina* types only. This is in accordance with the general character of the associations of the *ampliapertura* to *ciperoensis*-zones, of Oligocene age, of Trinidad.

There is a distinctly different faunal composition in the overlying sediments, such as those of Burcht near Antwerp, Dingden, Woltrup, Twistringen and the Peel borings, which are considered to be all of Miocene age. They represent several rock-stratigraphic units. The associations are most distinct and most varied in the Peel borings. These borings also show that the change in the planktonic fauna is fairly abrupt (fig. 3), just as it is in the benthonic associations (Ten Dam and Reinhold, 1942; Indans, 1958). Some of the general types of the underlying beds (*Globigerina globularis*, *G. bulloides*, *G. obesa*) continue their range, but there is the sudden appearance of *Globigerinoides triloba*, *Globigerina* cf. *nepenthes*, *G. ciperoensis* and *Globorotalia* cf. *scitula*. There are as yet no possibilities for a subdivision of our Miocene sediments by means of planktonic Foraminifera.

The Miocene associations as a whole do not fit in with the Trinidad succession. *Globigerina ciperoensis* is characteristic of the *ampliapertura* to *ciperoensis*-zones of the Oligocene of this island, whereas *Globigerinoides triloba* and the *Globorotalia fohsi* group are not present below the *dissimilis*-zone, which is of mainly Miocene age. This is a caution against overestimating the time-stratigraphic value of planktonic types. For our area, if we somewhat extend the range of *G. ciperoensis* on the assumption that we are dealing with late descendants in the remote North Sea, the age of the associations would agree best with that of the *dissimilis*-zone s.l. of Trinidad. According to Drooger (1956) the age of this zone in its original wide sense ranges from at least Aquitanian into Helvetian.

Although the entrance of our new Miocene planktonic species through the "Channel" is the most plausible, the sudden appearance of *Globigerinoides triloba* and *Globorotalia* cf. *scitula* or nearly identical *Globorotalia* types in the North Sea basin, the Vienna basin and Moravia might point to close marine connections between these regions. Kautsky (1927) also mentioned the possibility of such connections because of the resemblance of the molluscan fauna of Hemmoor with faunae of the Vienna basin. In the faunae of benthonic Foraminifera of Central Europe similar resemblance may be found with those of the North Sea basin. In Central Europe (Buday and Cicha, 1956; Papp, 1958) the *Globigerinoides-Globorotalia* associations are of Tortonian age and they include *Orbulina*, a genus absent in the North Sea deposits. Possibly *Orbulina* did not yet exist at the moment of the Miocene invasion in our area, but its absence might also be due to unknown factors. The former supposition would mean that the lower part of our Miocene sediments of the North Sea basin would be older, of at least Helvetian age.

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ISOLATION, CHARACTERIZATION, AND STRUCTURAL STUDIES ON SIX ALKALOIDS FROM LUNASIA AMARA BLANCO

BY

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(Communicated by Prof. Dr. P. E. VERKADE at the meeting of May 30, 1959)

§ 1. *Introduction*

In the present paper we intend to report on results obtained in the course of our investigation of the alkaloid constituents of the bark of *Lunasia amara* Blanco. In this connection it would seem appropriate first of all to mention a few historical data.

In the Malay Archipelago and in the Philippines there are found four species of the *Lunasia* genus: *L. costulata* (Miquel), *L. parvifolia* (Planch.), *L. grandifolia* (Miquel), and *L. amara* (Blanco), belonging to the *Rutaceae*. KOORDERS and VALETON proposed to regard these species merely as variations of *Lunasia amara* Blanco, since the only difference consists in the size of the leaves, while the flowers and fruits are identical [1]).

The name *Lunasia amara* is probably derived from *lunas*, indicating an antidote, and *amara*, which means bitter [2]).

In the northern part of Australia a species of the *Lunasia* genus occurs, which is considered to be identical with the New Guinea species *L. quercifolia* (Warb.) Lauterb. et K. Schum. *L. quercifolia* is closely related to *L. amara*, not only morphologically, but apparently also in physiological respects, since it appears from recent investigations that the alkaloid pattern resembles that of *L. amara* [3]).

L. amara has been extensively examined by BOORSMA [4]). This worker reports that in some Java markets the bark of *Lunasia* used to be sold because of medicinal properties ascribed to extracts of it. Several other authors mention the application in the Philippines of *Lunasia* extracts as arrow poison and some of them attribute the pharmacological effects to digitalis-like glycosides [5]). The latter, however, is doubtful. It now seems probable that the remarks in question relate to *Lophopetalum toxicum*; a consignment of this to various European museums was mislabelled

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1) S. H. KOORDERS and T. VALETON, Bijdrage No 4 tot de kennis der boomsoorten op Java, Mededeelingen uit 's Lands Plantentuin te Buitenzorg **17**, 226 (1896).

2) Compare *e.g. l.c.* 8.

3) R. JOHNSTONE, J. R. PRICE, and A. R. TODD, Australian J. Chem. **11**, 562 (1958).

4) *a.* W. G. BOORSMA, Over het vergiftig bestanddeel van *Lunasia costulata* Miq., Mededeelingen uit 's Lands Plantentuin te Buitenzorg **31**, 13, 126 (1899).
b. W. G. BOORSMA, Ueber philippinische Pfeilgifte, Bull. Inst. Bot. Buitenzorg **6**, 14 (1900).
c. W. G. Boorsma, Bull. Inst. Bot. Buitenzorg **21**, 8 (1904).

5) *a.* P. C. PLUGGE, Nederland Tijdschr. Geneesk. **1896**, II, 132.
b. Further references are given by H. DIETERLE and H. BEYL, *l.c.* 10.

Lunasia. This mistake was discovered by BOORSMA, and the botanical confusion that had arisen from it was cleared up by him. To this it may be added that BRILL and WELLS [6]) later stated they had found no evidence for the use of *Lunasia* extracts as arrow poison. More than two decennia afterwards, however, these erroneous reports consciously induced STELDT and CHEN [7]) to examine *L. amara* for constituents with digitalis-like properties; the latter naturally were not found.

BOORSMA [4]) was the first investigator to isolate crystalline alkaloids from *L. amara*. From the bark he recovered lunasine (m.p. 143°), lunacrine (m.p. 87–88°; after drying at 105°, however, the m.p. was 114°), and lunacridine (m.p. 82–84°); from the leaves BOORSMA recovered yet another alkaloid, which he called lunine (m.p. 219°).

WIRTH [8]) isolated and characterized lunacrine, but failed to find lunacridine; a compound with m.p. 188–189° was called lunasine by him [9]).

DIETERLE and BEYL [10]) partly succeeded in isolating the alkaloids mentioned by BOORSMA and in extending the chemical investigations of WIRTH somewhat further.

STELDT and CHEN [7]) isolated four alkaloids: lunacrine and lunacridine, as mentioned by BOORSMA; lunamarine, probably identical with the compound which DIETERLE and BEYL had called lunacridine and which is different from BOORSMA's lunacridine, and a new alkaloid, which they called lunamaridine. The said workers could not find either BOORSMA's lunasine or his lunine.

After a long period of apparent inactivity two groups of workers recently reported on the alkaloids of *Lunasia*.

GOODWIN, SMITH, and HORNING [11]) isolated from the leaves of *Lunasia amara*, originating from the Philippines, a hitherto unknown alkaloid, whose identity with 4-methoxy-2-phenylquinoline as synthesized by them could be ascertained by comparison. In addition other alkaloids, including (–)-lunacrine, were isolated [12]), a fact to be discussed in part later in this paper.

From the bark of *L. quercifolia*, occurring in northern Australia, JOHNSTONE, PRICE, and TODD [3]) recovered a previously unknown alkaloid 1-methyl-2-phenyl-7-methoxy-4-quinolone. The proof of the structure of this latter compound is based, apart from a number of degradation reactions, on a direct comparison with material synthesized by the said workers. JOHNSTONE, PRICE, and TODD further isolated three more alkaloids, which they assumed to be identical with BOORSMA's lunacrine, lunacridine, and lunine respectively. We shall revert to these discoveries later.

Some of the results obtained by the two last-mentioned groups of workers enabled GOODWIN and HORNING to determine the structural formulae of lunacrine and lunacridine [13, 14]); these will be discussed later in this paper.

In the above account of the older investigations we have not entered into data contradicting each other, as in the case of statements about

[6]) H. C. BRILL and A. H. WELLS, Philippine J. Sci. **12A**, 180 (1917).

[7]) F. A. STELDT and K. K. CHEN, J. Am. Pharm. Assoc. Sci. Ed. **32**, 107 (1943).

[8]) E. H. WIRTH, Studiën over *Lunasia amara* (Blanco) var. *costulata* (Hoch.), Thesis Leiden (1931); Pharm. Weekblad **68**, 1011 (1931); C. A. **26**, 557 (1932).

[9]) Pharmacognostic details of WIRTH's alkaloids are given by F. AMELINK, Pharm. Weekblad **69**, 1390 (1932).

[10]) H. DIETERLE and H. BEYL, Arch. Pharm. **275**, 174, 276 (1937).

[11]) S. GOODWIN, A. F. SMITH, and E. C. HORNING, J. Am. Chem. Soc. **79**, 2239 (1957).

[12]) Private communication by Dr. S. GOODWIN; compare also *l.c.*11.

[13]) S. GOODWIN and E. C. HORNING, J. Am. Chem. Soc. **81**, 1908 (1959).

[14]) S. GOODWIN, J. N. SHOOLERY, and L. F. JOHNSON, J. Am. Chem. Soc. **81**, in press (1959).

melting points, optical rotations, and empirical formulae of the alkaloids. When we started our investigation, our first objective was to carry out this characterization properly before commencing the structural investigation of the *Lunasia* alkaloids; indeed, nothing was known with certainty about their chemical structure when seven years ago we began our research.

The recent papers of the two last-mentioned groups of workers induced us to submit this preliminary report about our similar investigations.

§ 2. *Isolation and characterization of the alkaloids (with the cooperation of Mr. J. S. Bontekoe)*

In compliance with the requests made by the former of the present authors, through the good offices of the Royal Tropical Institute of Amsterdam, to the Forest Research Institute, Bogor (Java, Indonesia), bark of *Lunasia amara* Blanco was twice collected in the southern part of Jogjakarta (Java) under the auspices of the Forestry Management of Jogjakarta. The first collection, made in March 1952, yielded 1.3 kg of air-dried bark (batch 1); the second collection, made about February–March 1953, yielded about 17 kg of air-dried bark (batch 2).

Batch 1 was worked up and examined chemically, by way of a first orientation, with the cooperation of Mr. J. S. Bontekoe in the Laboratory of Organic Chemistry of the University of Amsterdam. Batch 2 was

TABLE A

Some properties of the *Lunasia amara* alkaloids

Alkaloid	Melting point	$[\alpha]_D^{alcohol}$	Formula *	Functional groups *	Fluorescence under U.V. light	Remarks
I	230–233°	inactive	$C_{18}H_{15}NO_4$	1 —OMe 1 >NMe	very strong	weakly basic
II	201–203°	— 14°	$C_{16}H_{19}NO_4$	1 —OMe 1 >NMe	strong	hydrochloride m.p. 201–202°
III	107–109°	slight or none	$C_{17}H_{23}NO_4$(?)	"nearly" 2 —OMe 1 >NMe	weak	very weakly basic
IV ±)–Lunacrine	145–146°	inactive	$C_{16}H_{19}NO_3$	1 —OMe 1 >NMe	strong	forms hydrochloride
V (+)–Lunacridine	79– 81°	+ 30°	$C_{17}H_{23}NO_4$	2 —OMe 1 >NMe	weak	very weakly basic
VI	in the range of 80–90°	— 45°	$C_{16}H_{19}NO_3$	1 —OMe 1 >NMe	weak	very weakly basic

* The authors are grateful to Messrs P. J. Hubers, Amsterdam, M. van Leeuwen Delft, and W. Manser, Zürich, for these analyses.

extracted by N. V. Organon, Oss, and worked up further by us here, at first with the cooperation of Mr. E. J. 's-GRAVENMADE. In the following discussion we shall differentiate between the two batches only in those cases where there was an essential difference in method of treatment.

In the treatment of the plant material we employed commonly used methods, such as extraction of acid, neutral, and alkaline extracts with lipoid solvents and chromatography with the aid of aluminium oxide.

Six crystalline alkaloids obtained in this way are for the present referred to as Lunasia I–VI. Data about them are listed in Tables A (miscellaneous data), B (ultra-violet spectral data), and C (infra-red spectral data, obtained by Mr. A. VAN VEEN).

In this connection it has to be mentioned that we have so far failed to find in our extracts the alkaloids 4-methoxy-2-phenylquinoline and

TABLE B

Ultra-violet spectral data of *Lunasia amara* alkaloids *

Alkaloid	λ max, mμ	log max	λ min, mμ	log min
I	247	4.47	230	4.34
	289	4.05	283	4.02
	338	4.08	304	3.92
	352	4.06	344	4.04
II	217	4.20	223/224	4.16
	243	4.63	272	3.44
	300	3.82	303	3.81
	321	3.99		
	331/332	3.93		
III	239	4.30	246	4.22
	257/258	4.33	275/277	3.82
	284	3.85	310	3.22
	334	3.45		
IV	219/220	4.32	225	4.30
(±)–Lunacrine	241	4.61	269/270	3.58
	299/300	4.00	306	3.96
	312/313	4.03	319/320	3.93
	325	3.97		
V	239	4.36	246	4.30
(+)–Lunacridine	258	4.40	275/277	3.86
	285	3.91	311/312	3.37
	333	3.53		
VI	219	4.34	224	4.29
	239	4.47	274	3.73
	300	3.94	317/318	3.51
	325	3.54		

* Measurements were made in alcohol; only the most conspicuous maxima and minima are mentioned.

1-methyl-2-phenyl-7-methoxy-4-quinolone respectively, obtained by GOODWIN *et al.*[11]) and by JOHNSTONE, PRICE, and TODD [3]). With a view to this we also examined our material with the aid of paper chromatography, in which case we used the two last-mentioned compounds as test substances; these were partly prepared by a method different from the published one.

TABLE C

Infra-red spectra of *Lunasia amara* alkaloids

Lunasia alkaloid	Main absorption bands (s = strong, m = medium, w = weak, br = broad). Measurements in chloroform solutions
I	—— 2980 m 1628 w 1600 s 1577 m 1486 s 1456 m 1439 m 1398 w 1368 w 1344 w 1306 m 1276 m 1250 s 1159 w 1106 w 1070 m 1040 m 939 m 911 w 850 w 816 m
II	3610 w 3300 m (OH) 2980 s 1617 m 1603 m 1582 s 1546 ss 1504 s 1452 s 1416 w 1386 w 1373 w 1347 w 1250 s 1174 m 1130 s 1075 s 1012 w 964 m 952 m 894 w 818 m
III	—— 2980 m 1642 s 1597 s 1466 s 1410 w 1365 s 1299 m 1143 m 1103 m 1075 s 1044 w 998 m 986 w 919 w 912 w 888 w 844 w
IV (±)–Lunacrine	—— 2980 s 1620 s 1594 s 1551 ss 1512 s 1468 s 1436 m 1390 m 1373 s 1266 s 1176 m 1122 m 1072 s 978 m 947 s 830 m
V (+)–Lunacridine	3300(m,br)(OH) 2985 s 1633 s 1614 w 1587 s 1468 s 1414 w 1368 s 1297 m 1242 s 1139 m 1110 w 1075 s 1010 w 988 m
VI	—— 2985 s 1660 s 1626 s 1600 m 1574 m 1495 m 1470 s 1418 m 1402 m 1364 m 1287 w 1252 s 1159 m 1072 s 1017 m 934 m

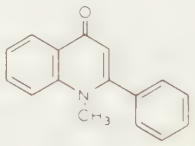
§ 3. *Discussion of the alkaloids, with the exception of Lunasia II (with the cooperation of Mr. A. van Veen)*

A comparison of the empirical formulae of our alkaloids I-VI (Table A) among each other and with the 4-methoxy-2-phenylquinoline ($C_{16}H_{13}NO$) isolated by GOODWIN *et al.*[11]) as well as with the compound 1-methyl-2-phenyl-7-methoxy-4-quinolone ($C_{17}H_{15}NO_2$) obtained by JOHNSTONE, PRICE, and TODD [3]) makes it possible to classify the *Lunasia* alkaloids according to their relative hydrogen content into *two groups*, one containing less and the other more hydrogen. The alkaloids lunamarine ($C_{18}H_{15}NO_4$, m.p. 245–246°) and lunamaridine ($C_{16}H_{15}NO_2$, m.p. 209–210°) isolated by STELDT and CHEN [7]) would belong in the first category.

These alkaloids containing relatively little hydrogen are optically inactive.

1) *Lunasia I.*

In view of its empirical formula Lunasia I ($C_{18}H_{15}NO_4$), along with the said alkaloids of a known structure, $C_{16}H_{13}NO$ and $C_{17}H_{15}NO_2$, both to be regarded as derived from 2-phenyl-4-quinolinol, would belong to the group of compounds containing relatively little hydrogen. It is striking that the empirical formula of Lunasia I ($C_{18}H_{15}NO_4$) from *L. amara* agrees with that of 1-methyl-2-phenyl-7-methoxy-4-quinolone ($C_{17}H_{15}NO_2$) isolated from *L. quercifolia*, with an *extra* methylene-dioxy group. Lunasia I in the colour reaction for the methylene-dioxy group with the aid of phloroglucinol and sulphuric acid [15]) gave a clearly positive result; this reaction was negative in the case of Lunasia II-VI. For Lunasia I – also in view of the quantitative determination of one methoxyl and one methylimide group (Table A) - the following structural formula might be suggested:



plus one *methoxyl* and one *methylene-dioxy* group.

Fig. 1, Lunasia I

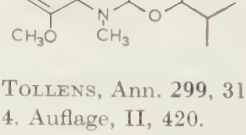
We are engaged in the further determination of the structure.

2) *Lunasia IV, (±)–lunacrine*

On the basis of the elementary analysis we assign to our optically inactive Lunasia IV with m.p. 145-146° the empirical formula $C_{16}H_{19}NO_3$; the quantitative determinations of the functional groups point to the presence of one methoxyl as well as one methylimide group.

A direct comparison of the infra-red spectrum (in carbon disulphide) of Lunasia IV with the spectrum of (–)–lunacrine (m.p. 117–118°, after drying) isolated by Johnstone, Price, and Todd [3]) – which comparison was performed by Dr. J. R. Price – as well as a comparison with optically active lunacrine obtained by Goodwin, Smith, and Horning – which comparison was performed by Dr. S. Goodwin – showed that these spectra are essentially identical. Lunasia IV is therefore identical with the racemate corresponding to the optically active lunacrine of these two groups of workers.

For lunacrine the following structure (a linear dihydrofuranoquinolone) has been indicated by Goodwin *et al.*[13,14]) on the basis of chemical and physical data, including nuclear magnetic resonance (NMR) spectra:



Lunasia IV, (±)–lunacrine

Fig. 2

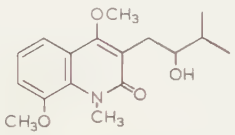
[15]) K. Weber and B. Tollens, Ann. **299**, 317 (1898); Houben–Weyl, Methoden der organischen Chemie, 4. Auflage, II, 420.

3) *Lunasia V, (+)-lunacridine.*

To this dextro-rotatory compound, which we isolated only from batch 1, we assign the composition $C_{17}H_{23}NO_4$; the determinations of the functional groups point to the presence of two methoxyl groups and one methylimide group. A broad band in the infra-red spectrum at 3300 cm^{-1} (Table C) clearly indicates the presence of a hydroxyl group.

A direct comparison of the infra-red spectrum of Lunasia V (in carbon disulphide) with the spectrum of optically active lunacridine (m.p. 85–86°), isolated by JOHNSTONE, PRICE, and TODD – which comparison was performed by DR. J. P. PRICE – proved the identity of Lunasia V with PRICE's (+)-lunacridine.

Chemical and spectral evidence obtained by GOODWIN and HORNING [13]), as well as by JOHNSTONE, PRICE, and TODD [16]), lead to the following structural formula (derivative of 4-hydroxy-2-quinolone):



Lunasia V, (+)-lunacridine

Fig. 3

Our above-mentioned analytical and spectral data as well as the observation of the low basicity of Lunasia V, which exhibits practically the same ultra-violet spectrum *e.g.* in alcohol and in 1 *N* alcoholic hydrochloric acid respectively, are in agreement with this.

4) *Lunasia III*

The results of the elementary analyses performed with Lunasia III indicate a composition $C_{17}H_{23}NO_4$, *i.e.* an empirical formula identical with that Lunasia V (lunacridine). The ultra-violet spectrum of III is practically identical with that of V, and the infra-red spectrum too shows a clear resemblance to that of V.

However, there are also remarkable differences between III and V, *e.g.* in melting point and optical activity. The most striking difference, however, is the absence in the infra-red spectrum of an absorption in the range from 3600 to 3000 cm^{-1}, whereas Lunasia V (lunacridine) does exhibit a hydroxyl absorption in this range.

In view of the fact that only a small quantity of Lunasia III was at our disposal, so that the purification may have been imperfect, we prefer to refrain for the present from drawing further conclusions with regard to the structure of Lunasia III.

5) *Lunasia VI*

Lunasia VI could be recovered only from batch 2; in this connection we would recall that we found Lunasia V exclusively in batch 1. Since

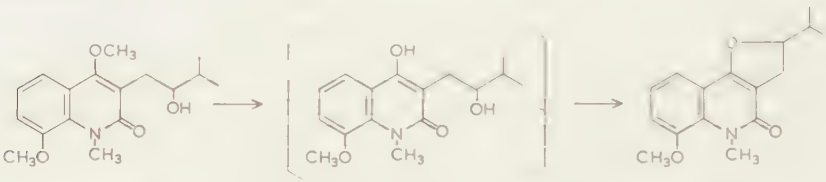
[16]) Compare J. R. PRICE in A. ALBERT (editor), Current Trends in Heterocyclic Chemistry, Butterworths Scientific Publications, London (1958), 92–99.

batch 2 had been subjected to a fairly prolonged treatment with hot 3 *N* hydrochloric acid, in contrast with batch 1, we suspected that during the second treatment a conversion of V (lunacridine) into VI might have taken place, *i.e.*:

$$C_{17}H_{23}NO_4 \text{ (V)} \rightarrow C_{16}H_{19}NO_3 \text{ (VI)} + CH_4O.$$

Actually a sample of V, (+)-lunacridine, could be converted by a treatment with hot 3 *N* hydrochloric acid into (−)-Lunasia VI in a yield of about 50 %.

Assuming the correctness of the structure for Lunasia V (lunacridine) discussed above and given once more below, and also in view of the properties of Lunasia VI, the following transformation V → VI seems plausible:



(+)-Lunasia V Fig. 4 (—)-Lunasia VI

This formulation is supported by the analogous reaction performed by Tatsuo Ohta and Yo Mori [17]) as given below:

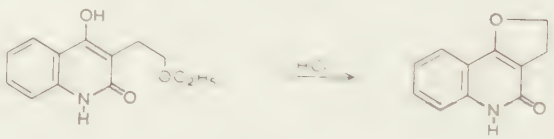
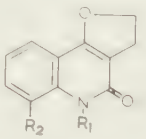


Fig. 5

In this connection it is to be noted that Price reported that from lunacridine (V) he had obtained lunacrine (IV), in poor yield, by the side of a main product with a lower melting point and much more weakly basic.[16]) This main product was not identified any further, but Price did give as his opinion that this might be the angular (*i.e.* dihydrofurano-2-quinolone) isomer of lunacrine (IV).[16])

Our above mentioned formula of VI is corroborated by the resemblance of the ultra-violet spectrum of Lunasia VI to that of a number of model substances of the following type [18]):



R_1 = H or methyl
R_2 = H or methoxyl

Fig. 6

[17]) Tatsuo Ohta and Yo Mori, Proc. Japan. Acad. **32**, 769 (1956).

[18]) The N-methyl compounds were prepared by methylation of the compounds (R_1 = H) which were prepared by us according to the syntheses described by M. F. Grundon, N. J. McCorkindale, and M. N. Rodger, J. Chem. Soc. **1955**, 4284.

One of these compounds, in particular, (R_1 = methyl, R_2 = methoxyl; m.p. 144–145°) – *i.e.* with a structure identical with that assigned above to VI, but without the isopropyl side-chain – possessed an ultra-violet spectrum strikingly similar to that of VI. *The infra-red absorption spectrum of the compound in question was from* 1680 *to* 1400 cm^{-1} *practically identical with the spectrum of VI* (Table C).

Dr. S. Goodwin kindly took a NMR spectrum of Lunasia VI (in $CDCl_3$). The NMR spectrum clearly shows, among other things, the presence of an isopropyl group.

§ 4. *Some structural investigations on Lunasia II (with the cooperation of Mr. A. van Veen).*

As far as we are aware, the laevo-rotatory Lunasia II, to which we assign the composition $C_{16}H_{19}NO_4$, has not been described by other workers. The determinations of the functional groups point to one methoxyl group and one methylimide group. The ultra-violet spectra of Lunasia II in neutral as well as in acid medium have a striking resemblance to those of Lunasia IV (lunacrine), but undoubtedly are not identical with them. The empirical composition of Lunasia II ($C_{16}H_{19}NO_4$) is identical with that of Lunasia IV or lunacrine ($C_{16}H_{19}NO_3$) *plus* one oxygen atom. The infra-red spectrum indicates the presence of a hydroxyl group (absorptions at 3610 and 3300 cm^{-1}); these absorptions are not present in the spectrum of Lunasia IV.

To Lunasia II we assign the following *linear* dihydro*pyrano*-4-quinolone structure:

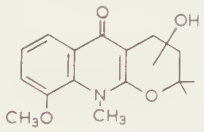


Fig. 7, Lunasia II

This structural formula is based by us, apart from the analytical data of course, on:

1. the identity, within the error of observation, of the ultra-violet spectrum of Lunasia II with the spectrum of the synthetic compound "B" to be mentioned hereinafter (§ 5);
2. the marked resemblance of the infra-red spectrum of Lunasia II (Table C) to that of the synthetic compound "B" to be mentioned in § 5 (*from* 1650 *to* 1240 cm^{-1} *practically identical*);
3. the optical activity of Lunasia II. In view of this the only possible positions for the OH group are in the pyran ring or on the *gem*-dimethyl group.

   In view of the difficulty of the esterification of the OH group as well as a (retro-pinacolinelike?) re-arrangement which II seems to undergo when heated with $KHSO_4$ (to form an *angular* "*furano* isomer"?), we would tentatively put the OH group in the α-position by the side of the *gem*-dimethyl group.

Dr. S. Goodwin kindly took a NMR spectrum of II (in $CDCl_3$). This spectrum, which shows, among other things, the absence of an isopropyl group as well as the presence of methyl groups – by the side of –OMe and >NMe, of course – according to Dr. Goodwin is compatible with the formulation for II suggested by us above.

It seems interesting to mention in addition that Lunasia II could be converted into an isomer (m.p. 202–203°) in a good yield by heating with 30 % sodium hydroxide solution. We assume this isomer to be the *angular* analogue of Lunasia II:

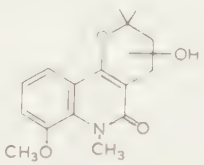
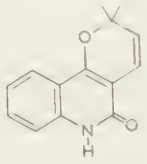


Fig. 8

in view of the very striking similarity of the ultra-violet and infra-red spectrum (*from* 1650 *to* 1350 cm^{-1} *practically identical*) to those of the synthetic compound "A" to be mentioned hereinafter (§ 5).

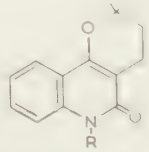
§ 5. *Synthesis of dihydroflindersine and some related compounds in connection with the structure of Lunasia II.*

The structure of the alkaloid flindersine occurring in the wood of *Flindersia australis*[19]) has been proved by Brown, Hobbs, Hughes, and Ritchie by means of degradation reactions[20a]) as well as by synthesis[20b]). In the course of their investigations these workers also obtained dihydroflindersine and N-methyldihydroflindersine (fig. 9).



Flindersine

Fig. 9



R = H, Dihydroflindersine
R = Me, N-Methyldihydroflindersine

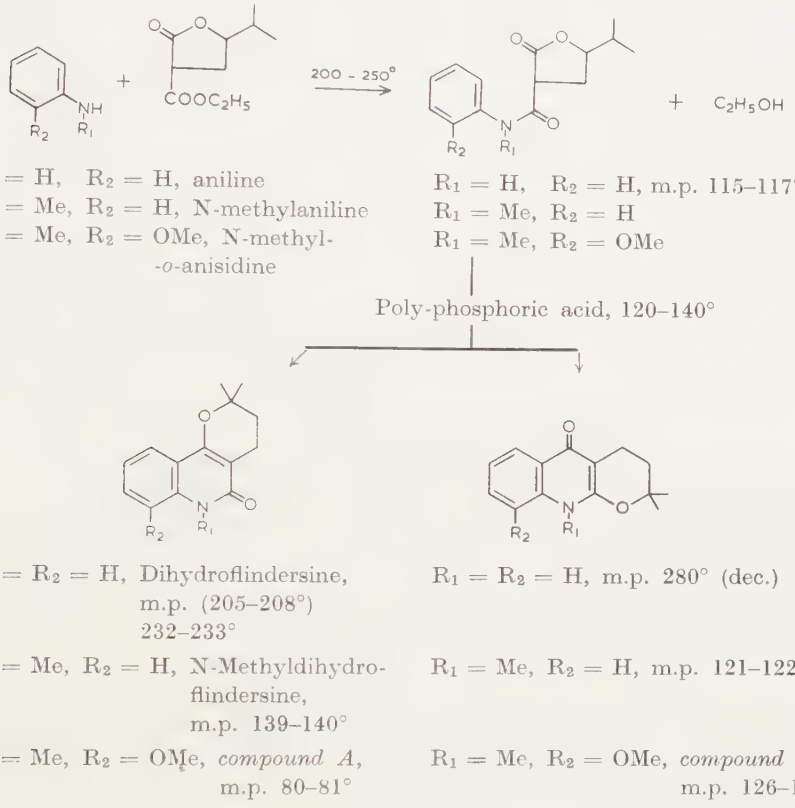
We synthesized the latter two compounds and some analogues in a different way, *viz.*, by causing γ-isopropyl-α-ethoxycarbonyl-γ-butyrolactone to react with *e.g.* aniline, N-methylaniline, N-methyl-*o*-anisidine according to the scheme given after this (fig. 10).

[19]) H. Matthes and E. Schreiber, Ber. deut. pharm. Ges. **24**, 385 (1914); C.A. **9**, 1661 (1915).

[20]) *a.* R. F. C. Brown, J. J. Hobbs, G. K. Hughes, and E. Ritchie, Australian J. Chem. **7**, 348 (1954);

b. R. F. C. Brown, the Late G. K. Hughes, and E. Ritchie, Australian J. Chem. **9**, 277 (1956).

The lactone in question (b.p. 113—115°/1 mm; n_D^{20} 1.4484) was obtained in a 65 % yield by the reaction of 1-chloro-3-methylbutane-2-ol with diethyl malonate, analogously to the preparation of the corresponding γ-ethyl compound by HELFERICH and SPEIDEL [21].



$R_1 = H$, $R_2 = H$, aniline
$R_1 = Me$, $R_2 = H$, N-methylaniline
$R_1 = Me$, $R_2 = OMe$, N-methyl-*o*-anisidine

$R_1 = H$, $R_2 = H$, m.p. 115–117°
$R_1 = Me$, $R_2 = H$
$R_1 = Me$, $R_2 = OMe$

$R_1 = R_2 = H$, Dihydroflindersine, m.p. (205–208°) 232–233°

$R_1 = R_2 = H$, m.p. 280° (dec.)

$R_1 = Me$, $R_2 = H$, N-Methyldihydroflindersine, m.p. 139–140°

$R_1 = Me$, $R_2 = H$, m.p. 121–122°

$R_1 = Me$, $R_2 = OMe$, *compound A*, m.p. 80–81°

$R_1 = Me$, $R_2 = OMe$, *compound B*, m.p. 126–127°

Fig. 10.

In these syntheses two products could invariably be isolated, which gave identical results in the elementary analyses and in the methoxyl and methylimide determinations, but whose solubilities in dilute acid differed.

In these cases we assumed that the soluble compounds would be the isomers with the linear structure and the insoluble compounds the angular isomers (lower basicity owing to the 2-oxo grouping).

A proof of the correctness of this assumption for two of the angular compounds ($R_1 = H$, $R_2 = H$, and $R_1 = Me$, $R_2 = H$) and at the same time a *proof of the presence of the pyran ring* in these compounds was given by a direct comparison (mixed melting point, infra-red spectra) with dihydroflindersine and N-methyldihydroflindersine respectively, obtained from Dr. E. RITCHIE.

On the analogy of the above, for compound "B" (soluble in dilute acid) the *linear* dihydro*pyrano*quinolone structure would follow. This view is

[21] B. HELFERICH and J. A. SPEIDEL, Ber. **54**, 2637 (1921).

corroborated by the observation that in spite of a high degree of similarity (*e.g.* basicity, ultra-violet spectrum, behaviour with paper chromatography), as may be expected in view of the close relationship of the structure assigned to it with that of (±)–lunacrine (linear dihydro*furano*-quinolone), compound "B" obviously is not identical with the latter, because of differences in melting point and in ultra-violet and infra-red spectra.

The resemblance of compound "B" to Lunasia II (fig. 7), and of compound "A" to the isomeric compound obtained from Lunasia II by a treatment with sodium hydroxide solution (fig. 8), has been discussed in § 4.

Acknowledgements

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Conclusions

1. From the bark of *Lunasia amara* Blanco of Javanese origin we obtained six crystalline alkaloids, hereinafter referred to as Lunasia I–VI.

The empirical formulae and some chemical and physical properties (*e.g.* optical rotations, ultra-violet and infra-red spectral data) of these alkaloids are given.

2. For Lunasia I, we tentatively propose the structure of a 1-methyl-2-phenyl-4-quinolone substituted by one methoxyl and one methylenedioxy group.

3. Lunasia IV was found to be identical with the racemate corresponding to the optically active alkaloid lunacrine isolated independently by two groups of other workers from the leaves of Philippine *L. amara*, and from the bark of Australian *L. quercifolia* respectively.

4. Lunasia V was found to be identical with (+)–lunacridine, also found in the bark of Australian *L. quercifolia* by other investigators.

5. It is shown that Lunasia VI is an artefact derived by acid treatment from Lunasia V, (+)–lunacridine.

On the basis of the formula assigned to lunacridine by other workers and on the basis of the aforementioned conversion, together with evidence about the structure of Lunasia VI obtained by us, a structural formula (2-isopropyl-5-methyl-6-methoxy-4-oxo-2, 3: 4, 5-tetrahydrofurano [3,2–*c*] quinoline) is assigned to Lunasia VI:

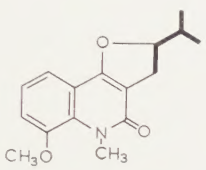

Fig. 11, Lunasia VI

6. The following structure (2, 2, 10-trimethyl-9-methoxy-5-oxo-3, 4: 5, 10-tetrahydro-2*H*-pyrano [2, 3–*b*] quinoline, substituted by a hydroxy group in the pyran ring or in the *gem*-dimethyl group) is assigned to Lunasia II:

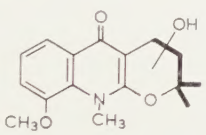

Fig. 12, Lunasia II

7. Syntheses of dihydroflindersine, N-methyldihydroflindersine, and some related compounds are reported in connection with the elucidation of the structure of Lunasia II.

8. It seems interesting with regard to alkaloid biosynthesis to note the presence of an "*isoprene*" unit in our dihydrofurano- and dihydropyrano-quinolone alkaloids (*e.g.* the formulae given above).

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